Vasoactive effects of erythropoietin on human placental blood vessels in vitro

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OBJECTIVE: The aim of this study was to investigate the direct effect of erythropoietin on human placental vessels.

STUDY DESIGN: Placental vessel rings (n = 8 for each group) from uncomplicated pregnancies were exposed to recombinant human erythropoietin (10-300 IU/mL) in an isometric myograph. One-way analysis of variance with the Bonferroni posttest was used to evaluate significant levels of differences.

RESULTS: Recombinant human erythropoietin evoked reproducible contractions on the vessel rings in a dose-dependent way, which were marked significantly more on veins than on arteries. These contractile responses were not changed by captopril (10^{-5} mol/L) but were blunted significantly by losartan (10^{-5} mol/L).

CONCLUSION: We concluded that recombinant human erythropoietin exerts a direct contractile effect on human placental vessels, angiotensin II type 1 receptors are needed to mediate these responses, and erythropoietin might participate in one of the humoral mechanisms that are involved in the control of the human placental vascular bed and also in the pathogenesis of intrauterine growth restriction and preeclampsia.
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Key words: Human placental blood vessel, erythropoietin, angiotensin II type 1 receptor, contractility

The main side effect of the chronic application of recombinant human erythropoietin (rHuEPO) as therapy for patients who receive hemodialysis has been reported to be the development or aggravation of hypertension, which can be explained only partially by an increase in blood viscosity,[1] a diminished hypoxic vasodilatation, or an enhanced cardiac output that is caused by a better level of myocardial oxygenation.[2] The development of hypertension directly after the application suggested that other pressor mechanisms may be involved.[3] The direct vasoconstrictor effect of erythropoietin has been reported by various authors in animal studies,[4,5] but we have found no data in the literature on the direct effect of erythropoietin on human placental blood vessels. The placental transfer of erythropoietin in humans seems quite unlikely,[6] but the human placenta[7] and the fetal liver, kidney, spleen, and bone marrow[8] produce erythropoietin during pregnancy. The erythropoietin receptor protein and messenger RNA, classically found in erythroid precursor cells,[9] have been described in other cell types, including endothelial cells of the fetoplacental vasculature.[10] These novel and nonclassic sites of erythropoietin and erythropoietin-receptor expression raise the possibility of physiologic roles for this hormone that are not related necessarily to erythropoiesis. Thus, the direct effects of rHuEPO on isolated human placental blood vessels were examined.

Considerable interest has focused recently on the potential for angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists to affect the response to erythropoietin.[11] The conractile effect on the rat mesenteric artery can be abolished by losartan (a selective antagonist of angiotensin II type 1 receptor [AT_1 receptor]) but cannot be blunted by captopril (an angiotensin-converting enzyme inhibitor).[4] In the current study, the effects of captopril and losartan on the rHuEPO-induced contractions of isolated human placental veins and arteries were also investigated.

Material and methods

Placentas were obtained from the delivery room of the Department of Obstetrics and Gynecology of the university immediately after the birth; they were transferred in 500 mL of icy Krebs-Henseleit buffer (118 mmol/L sodium chloride, 5 mmol/L potassium chloride, 2 mmol/L calcium chloride, 0.5 mmol/L magnesium sulfate, 1 mmol/L potassium sulfate, 25 mmol/L

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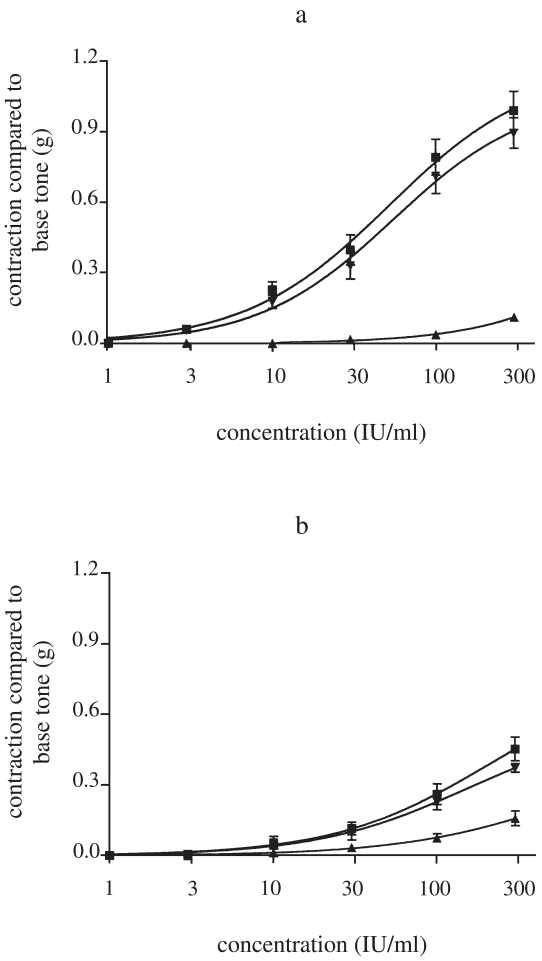

Figure. a, Semilogarithmic dose-response curve of rHuEPO for contractility of human placental veins and effects of captopril and losartan on contractions of human placental veins elicited by rHuEPO. rHuEPO evoked reproducible contractions in human placental veins in a significant and dose-dependent way. At all applied doses, effect of rHuEPO on placental veins was marked more significantly, compared with that on arteries. Incubation of vessel rings with captopril (10^{-5} mol/L) did not affect their contractile response to rHuEPO. In contrast, losartan at a concentration of 10^{-5} mol/L completely abolished contractile responses of vessel rings at 10 IU/mL rHuEPO and significantly blunted those responses at 300 IU/mL rHuEPO. *Closed squares,* Human placental veins + rHuEPO (n = 8); *closed inverted triangles,* human placental veins + rHuEPO + captopril (n = 8); *closed triangles,* human placental veins + rHuEPO + losartan (n = 8). **b**, Semilogarithmic dose-response curve of rHuEPO for contractility of human placental arteries and effects of captopril and losartan on contractions of human placental arteries elicited by rHuEPO. rHuEPO evoked reproducible contractions in human placental arteries in a significant and dose-dependent way. Incubation of vessel rings with captopril (10^{-5} mol/L) did not affect their contractile response to rHuEPO. In contrast, losartan at a concentration of 10^{-5} mol/L completely abolished contractile responses of vessel rings at 10 IU/mL rHuEPO and significantly blunted those responses at 300 IU/mL rHuEPO. *Closed squares,* Human placental arteries + rHuEPO (n = 8); *closed inverted triangles,* human placental arteries + rHuEPO + captopril (n = 8); *closed triangles,* human placental arteries + rHuEPO + losartan (n = 8).

sodium bicarbonate, 10 mmol/L glucose; pH-7.4); the experiments were begun 10 to 30 minutes after the birth. All the placentas originated from term pregnancies that ended with uncomplicated deliveries. The ages of the women from whom the placentas were obtained ranged between 19 and 28 years, with an average of 25.3 years. The gestational age at delivery was between 37 and 40 weeks, with an average of 38.1 weeks of gestation. After the umbilical cord had been cut off, thin polyethylene cannulas were led into the vein (with larger diameter) and the two arteries (with smaller diameters) in the stub, to separate the veins and arteries on the fetal surface of the placenta. The vessels were prepared for in vitro measurement according to the method outlined by Angus and Wright.[12] Rings that were 1 to 1.3 mm in diameter were dissected free from the identified veins and arteries just before they head towards the stem villi. Small-diameter resistance vessels, compared with conduit vessels, contribute more to the hemodynamics of placental bed perfusion, although the question of the location and the diameter of resistance vessels is unsolved, in general, and the feed arteries can be as active in blood flow control as the microvasculature.[13] The precise length of the rings (4 mm) was achieved by the use of a fixed double-bladed scalpel. The loose connective tissue was removed carefully under a cool fiber optic light source and binocular dissection microscope (magnification, ×10). The rings were taken distally from the site of introduction of the cannulas; the endothelium therefore remained intact.

The rings were mounted diametrically (as ring preparations) between two stainless steel wire hooks in the organ bath of an isometric myograph that contained 10 mL of Krebs-Henseleit buffer. The organ bath was maintained at 37°C and carbogen gas (95% oxygen + 5% carbon dioxide) was bubbled through it. After being mounted, the rings were equilibrated for 90 minutes before the experiment, and the buffer was changed every 10 minutes. The passive force was set at approximately 3.75 g and approximately 3.25 g for veins and arteries, respectively. The optimal degree of stretch was ascertained by a determination of a contraction versus passive force curve in response to a dose of 200 IU/mL of rHuEPO.[4]

The force of the vessel rings was measured with a gauge transducer (SG-02; Experimetria Ltd, London, UK), and was recorded by an ISOSYS Data Acquisition System (Experimetria Ltd). At our setting the passive force at the end of equlibration is termed the *base tone.* Each contractile response was measured as the difference between the top of the contraction curve and the base tone.

Measured points were plotted; curves were fitted to these points, and the data were statistically analyzed with Prism 2.01 software (GraphPad Software, San Diego, Calif). One-way analysis of variance with the Bonferroni posttest was used in all cases to evaluate the significance

levels of differences. Probability values that were lower than .05 were considered statistically significant.

Doses of rHuEPO (LaRoche, Budapest, Hungary) that ranged between 10 and 300 IU/mL were administered in a cumulative way. Each dose was given when the previous dose had exerted its maximal effect (within 2-10 minutes). Captopril (Sigma-Aldrich Ltd, Budapest, Hungary) and losartan (Merck Sharp & Dohme, Budapest, Hungary) were administered in a concentration of 10^{-5} mol/L 10 minutes before the first dose of rHuEPO.

Experimentation on human placentas has been approved by the local institution. Institutional review board approval was obtained May 9, 2000.

Results

rHuEPO evoked reproducible contractions in human placental blood vessel rings in a significant and dose-dependent way. At all applied doses, the effect of rHuEPO on the placental veins was marked more significantly compared with that on the arteries.

The vasocontractions in the case of the 10 IU/mL were 0.21 ± 0.03469 g and 0.05273 ± 0.01415 g for human placental veins and arteries, respectively. At the 300 IU/mL dose, the contractions were 1.088 ± 0.1296 g and 0.4509 ± 0.05017 g for the veins and arteries, respectively.

Incubation of the vessel rings with captopril (10^{-5} mol/L) did not affect the contractile response to rHuEPO. In contrast, losartan, at a concentration of 10^{-5} mol/L, completely abolished the contractile responses of the vessel rings at 10 IU/mL rHuEPO and significantly blunted them at 300 IU/mL rHuEPO (0.11 ± 0.01528 g and 0.1575 ± 0.03119 g for veins and arteries, respectively, Figure).

Comment

In the current study, rHuEPO was found to have a direct and dose-dependent contractile effect on human placental blood vessels in vitro. Because the fetoplacental vasculature lacks autonomic innervation,[14] the control of this vascular bed must involve humoral mechanisms; erythropoietin might participate in one of these mechanisms. To the best of our knowledge, the direct vasopressor effect of erythropoietin on human placental blood vessels has not been investigated yet. At all applied doses, the contractions that were elicited by erythropoietin were marked more significantly on veins than on arteries. These functional results confirmed the morphologic properties of the walls of the placental veins and arteries. The tunica media, which contains the force-producing smooth muscle cells, is thicker in placental veins than in arteries.[15] The contractile effect of rHuEPO on human placental blood vessels was abolished by losartan but was not blunted by captopril. Thus, it may be concluded that AT_1 receptors are needed to mediate the contractile response of human placental blood vessels to rHuEPO. There is a theoretic basis for this effect in view of the known close links between the renin-angiotensin system and erythropoiesis. It has been known for some time that the renin-angiotensin system is linked intricately with the production of endogenous erythropoietin in the peritubular fibroblasts of the kidney.[11] Additionally, renin substrate (angiotensinogen) has chemical similarities to erythropoietin.[16] All the components of the renin-angiotensin system have been shown to be present in the human term placenta.[17] The human term placenta contains predominantly AT_1 receptors with low levels of AT_2 receptors.[18] When placental membrane preparations were used, the AT_2 receptor antagonist PD123177 failed to compete for [^{3}H] angiotensin II binding at relevant concentrations, whereas the AT_1-receptor antagonist losartan competed in a monophasic manner.[19] Specific receptor binding sites for angiotensin II have also been identified in placental vascular smooth muscle cells.[20] Other experiments with the AT_1-receptor–selective antagonist losartan indicate that this subtype is responsible for most of the hemodynamic and cardiovascular effects of angiotensin II.[21] In the rat, rHuEPO exerts its primary action on vascular smooth muscle cells through an increase in angiotensin-receptor messenger RNA, which results in a parallel increase in angiotensin II receptor expression.[22]

The elucidation of these potential erythropoietin binding sites and the potential transport of erythropoietin across the placental barrier[23] are of clinical significance, concerning the assessment of the safety to the fetus of rHuEPO administration to anemic pregnant women. However, cases have been presented in which no maternal and perinatal complications that were attributable to rHuEPO were registered.[24] Because AT_1 receptor activation may play a role in preeclampsia[25] and AT_1 receptor expression is reduced in intrauterine growth restriction,[19] erythropoietin might also be involved, in part, in the pathogenesis of these disorders.

Our durrent findings add to the growing list of nonhematopoietic roles of erythropoietin during human development.

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