Acute hypoxia decreases plasma VEGF concentration in healthy humans

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Acute hypoxia decreases plasma VEGF concentration in healthy humans. Am J Physiol Endocrinol Metab 290: E434–E439, 2006. First published October 11, 2005; doi:10.1152/ajpendo.00508.2004.—Vascular endothelial growth factor (VEGF) is known to be upregulated by hypoxia in vitro. However, in vivo data about VEGF regulation in chronic hypoxic diseases are conflicting. We investigated the effects of hypoxia on plasma VEGF concentration in healthy subjects. To control known confounders, such as insulin, glucose concentrations, or exercise, hypoxic effects on VEGF were studied during experimentally clamping glucose concentrations at rest. In a double-blind crossover study design, we induced hypoxia for 30 min by decreasing oxygen saturation to 75% (vs. normoxic control) in 14 healthy men. Plasma VEGF concentration was determined at baseline, immediately after hypoxia had ended, and after a further 150 min. Levels of its soluble (s)Flt-1 receptor were assessed at baseline and at the end of the clamp. In parallel, catecholamine and cortisol levels were monitored. To investigate potential effects of glucose administration on the release of VEGF, we performed a third session, reducing glucose infusion for 30 min while serum insulin was held stable thereby inducing hypoglycemia. Hypoxia decreased VEGF levels compared with the normoxic control (P < 0.05). VEGF concentrations increased during hypoglycemia (P < 0.02) but were comparable to the normoglycemic control at the end of the clamp (P > 0.80). sFlt-1 receptor concentration remained unchanged during hypoxia and hypoglycemia compared with control (both P > 0.4). Epinephrine concentration (P < 0.01) increased upon hypoxia, whereas norepinephrine and cortisol did not change. Contrary to in vitro studies, in healthy humans hypoxia decreases plasma VEGF concentration, suggesting that systemic VEGF concentration may be differently regulated than the expression on cellular basis.

vascular endothelial growth factor; soluble Flt-1 receptor; in vivo; cortisol; catecholamines; clamp

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) is known to have numerous effects in metabolism and plays a role in oxygen regulation. However, which function VEGF circulating in systemic blood exerts and its potential clinical relevance is an open issue. In fact, altered systemic levels of VEGF have been reported in various pathological conditions, e.g., tumor growth (13), coronary artery disease (8), and chronic hypoxic diseases, possibly related to oxygen regulation. These observations suggest an involvement of VEGF in the pathomechanism of such diseases. Although VEGF has been demonstrated in various types of cells to be stimulated by hypoxia (5), in respiratory diseases with chronic hypoxic manifestation, data about VEGF regulation are conflicting. Several investigations

in obstructive sleep apnea (10, 15, 43, 46) and cystic fibrosis (29) revealing an increase in serum VEGF have been criticized, because VEGF is released from activated platelets during the in vitro clotting process (12, 17, 18). On the other hand, measuring VEGF in plasma (22) and in bronchoalveolar lavage fluid (31) yielded the same result. In other respiratory diseases, such as idiopathic pulmonary fibrosis, sarcoidosis (20, 31), or emphysema (19, 40), VEGF was decreased compared with healthy controls, whereas in smokers and moderate chronic obstructive pulmonary disease (COPD) it was increased (40). However, all of these data gained from patients may be biased by comorbidities known to influence VEGF, such as essential hypertension (1, 26), insulin resistance (2), or medications like statins (25), which calls into question a sole causal relationship between hypoxia and VEGF alteration. Also, high-altitude studies with healthy subjects remained inconclusive, with some authors reporting on enhancing effects of hypoxic conditions on VEGF blood concentration (42, 47), whereas others failed to find any change (27, 36) or even found a decrease (11). The variance in results is probably based on differences in conditions during the studies.

We examined the effects of acute hypoxia (vs. normoxia) on VEGF plasma levels in healthy young men, intending to avoid various influencing factors on VEGF concentration. Additionally, we also measured soluble VEGF receptor 1 (sFlt-1), which is known to be upregulated by hypoxia (9) and to bind VEGF with high affinity (39). The other important VEGF receptor, Flk-1/KDR (VEGF receptor 2), was not measured, since it is not upregulated by hypoxia and binds with distinctly lower affinity than the Flt-1 receptor (9).

Our results show that systemic hypoxia decreases VEGF levels compared with the normoxic control, during which VEGF concentrations increased probably due to circadian rhythm (21). Nevertheless, to rule out the possibility that changes observed during the normoxic control condition were induced by the administration of glucose or insulin, in a separate condition we reduced glucose infusion for 30 min while serum insulin was held stable during normoxic conditions.

METHODS

Subjects. Experiments were performed including 14 healthy white men aged 20–25 yr. Exclusion criteria were obesity (body mass index >25 kg/m²), chronic or acute illness (particularly respiratory diseases), alcohol or drug abuse, smoking, competitive sports, exceptional physical or mental stress, and current medication of any kind.

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Each participant gave written informed consent, and the study was approved by the University of Luebeck’s ethics committee.

Experimental design. Subjects participated in a hypoxic and a normoxic session separated by an interval of ≥4 wk. Hyperinsulineemic Euglycemic clamps were performed during the induction of hypoxia (and normoxia). The order of conditions was balanced across subjects, and experiments were performed in a double-blind fashion. All subjects were requested to abstain from alcohol, not to perform any kind of exhausting physical activity, and to go to bed no later than 2300 on the day preceding the test. On the days of experimental testing, subjects reported to the medical research unit at 1100 after an overnight fast of ≥12 h. Experiments took place in a sound-attenuated room with the subjects lying on a bed with the trunk in an almost upright position (~60°). A cannula was inserted into a vein on the back of the hand, which was placed in a heated box (50–55°C) to obtain arterialized venous blood. A second cannula was inserted into an antecubital vein of the contralateral arm. Both cannulas were connected to long, thin tubes, which enabled blood sampling and adjustment of the rate of dextrose infusion from an adjacent room without the subjects’ awareness.

Baseline blood samples for determination of plasma VEGF, sFlt-1, serum cortisol, epinephrine, and norepinephrine were collected. After a 1-h baseline period, insulin (H-insulin; Hoechst, Frankfurt, Germany) was infused at a continuous rate of 1.5 mIU·min⁻¹·kg⁻¹. A 20% dextrose solution was simultaneously infused at a variable rate to control plasma glucose levels. Arterialized blood was drawn at 5-min intervals to measure plasma glucose concentration (Glucose Analyzer; Beckman Coulter, Munich, Germany). Plasma glucose was held stable between 4.5 and 5.5 mmol/l, and dextrose infusion rates were determined continuously across consecutive 5-min intervals.

After 3 h (to reach a state of equilibrium between insulin and glucose concentration), hypoxia was induced for 30 min by decreasing oxygen saturation to 75%. On the normoxic control condition, oxygen saturation was held at the normal range of 96%. During induction of hypoxia and also during the normoxic control period, participants breathed through a tightly fitting face mask connected to a Tranjan 808 fresh gas supply (Draeger Medical Technology, Luebeck, Germany), using a valveless high-flow system between 14 and 17 l/min. Inspired oxygen fraction (F(O2)) was varied by adjusting oxygen and nitrogen.

Oxygen saturation was continuously measured by pulse oxymeter and was decreased to 75% within ~5 min. Blood samples for determination of cortisol, epinephrine, and norepinephrine were collected every 7.5 min between onset of hypoxia (normoxia) and 30 min after intervention period. After 30 min, oxygen saturation was quickly normalized. During the remaining 2-h period, blood samples were collected every 30 min. Plasma VEGF concentration was determined at baseline, after hypoxia had ended, and at the end of the clamp. Levels of its sFlt-1 receptor were determined at baseline and after 6 h of the clamp because this was the point of time when hypoxia-induced alterations in VEGF concentration showed a peak.

During the hypoglycemic clamp session, the experimental setup (including the face mask) was analogous to that of the hypoxic/normoxic intervention. The participants were blinded for the procedure by informing them that results of either the hypoxic or normoxic intervention were to be replicated during hypoglycemic conditions. After a 1-h baseline period, insulin was infused at a continuous rate of 1.5 mIU·min⁻¹·kg⁻¹. A 20% dextrose solution was simultaneously infused at a variable rate to control plasma glucose levels. After 2.5 h of euglycemia, hypoglycemia was induced for 30 min by lowering plasma glucose to 2.5 mmol/l; after another 30 min, plasma glucose was normalized again. The other procedures, including blood sampling throughout the session, were the same as in the first two conditions.

Assays. All blood samples were immediately centrifuged and the supernatants stored at −24°C until assay. VEGF and sFlt-1 probes were collected in synthetic plastic tubes containing ethylenediaminetetraacetate (EDTA), and plasma was separated within 30 min. Samples were measured by ELISA [VEGF interassay coefficient of variation (CV) 8.8%, intra-assay CV 6.7%; sFlt-1 interassay CV 7.7%, intra-assay CV 2.6%; R&D Systems, Minneapolis, MN]. The ELISA for VEGF determination measured exclusively unbound VEGF, without any cross-reactivity with bound VEGF. The ELISA for the VEGF receptor detected sFlt-1 as well as VEGF-sFlt-1 complexes. Assays for measurement of catecholamines and cortisol have been previously described (35).

Statistical analysis. Values are presented as means ± SE. Statistical analysis was based on analysis of variance for repeated measurements, including the factors “session” (hypoxia vs. normoxia) and “time” (time points of data collection). The interaction effect of these two factors was termed session × time. Paired t-tests were performed to compare hormone concentrations at particular points of time. A P value of <0.05 was considered significant.

RESULTS

Oxygen saturation, serum insulin, and plasma glucose. Upon induction of hypoxia, oxygen saturation decreased over a period of ~10 min and then stayed at a plateau of 74 ± 2%. Oxygen saturation during the corresponding period remained stable under the normoxic condition at a mean level of 98 ± 2% and during the hypoglycemic session at a mean level of 98 ± 1%. Determination of serum insulin and plasma glucose concentrations confirmed that respective blood levels also remained stable and did not differ between hypoxic and normoxic conditions (Fig. 1). During hypoglycemia, serum insulin concentrations were equal to those during the hypoxic/normoxic condition, whereas plasma glucose values decreased to a mean of 2.65 ± 0.06 mmol/l (Fig. 1).

VEGF and sFlt-1. Figure 2 summarizes VEGF plasma concentrations during the three conditions. VEGF levels decreased under hypoxia from baseline value to the end of the session (session × time P = 0.02). We analyzed the decrease during the hypoxic condition in relation to a gradual increase in VEGF levels during the control condition, which failed to reach significance (P = 0.54). This difference in VEGF concentrations between the conditions averaged, after baseline adjustment, 22.73% at the end of hypoxia and 27.35% at the end of the session. During hypoglycemia, plasma VEGF concentrations showed a peak.

![Figure 1. Mean (±SE) plasma glucose (circles) and serum insulin (triangles) concentrations during hypoxic (filled), normoxic euglycemic (gray), and hypoglycemic (open) conditions. Gray area marks time of hypoxic or hypoglycemic (vs. normoxic euglycemic) intervention. Arrows mark time points of vascular endothelial growth factor (VEGF) measurements.](image-url)
concentrations increased until the end of the intervention (session × time P = 0.012 hypoglycemia vs. control; P = 0.013 for a paired t-test at the end of hypoglycemia). Yet, this effect vanished at the end of the session (P = 0.89 for a paired t-test).

sFlt-1 receptor concentrations measured at baseline of the sessions and at the end did not indicate any distinct alteration following hypoxia or hypoglycemia (session × time P = 0.47 and P = 0.95, respectively; Fig. 3). However, Flt-1 receptor concentrations showed a marginally significant decrease at the end of the hypoxic (16.28%) and the control sessions (10.04%; effect of time P = 0.054). After the hypoglycemic intervention, Flt-1 receptor concentration was also decreased at the end of the session by 6.9% compared with baseline (P = 0.37; Fig. 3).

Blood pressure and neurohormonal stress response during hypoxic and control sessions. Systolic and diastolic blood pressure did not show a significant alteration during hypoxia compared with the control condition (session × time for both, P > 0.1; Fig. 4A). Cortisol and norepinephrine levels were also comparable between the two sessions (both P > 0.3). Epinephrine was significantly higher under the hypoxic compared with the control condition (P < 0.01; Fig. 4B). During hypoglycemia cortisol, epinephrine (Fig. 4B), and norepinephrine concentrations increased compared with the control session (P < 0.01 for all). Systolic blood pressure was unchanged (P = 0.07), and diastolic blood pressure decreased (P < 0.01; Fig. 4A).

DISCUSSION

The present results show that acute hypoxia decreases plasma VEGF concentration in healthy subjects at rest. This contrasts with in vitro studies showing an increased release of VEGF in response to hypoxia (6, 7, 32, 33, 44). Previous in vivo studies (11, 27, 36, 38, 42, 47) have so far provided mixed results, presumably reflecting the sensitivity of blood VEGF data to differences in the measurement procedure (16) and to influences of confounding circumstances such as glucose supply (3, 14, 30, 34, 41), insulin (32, 37), norepinephrine (7, 32), and cortisol secretion (24), and exercise (38). On this background, our study demonstrates for the first time that acute hypoxia decreases plasma VEGF in vivo when blood glucose and insulin concentrations are held stable and interference from exercise is precluded. Because we did not find any significant rise in norepinephrine or cortisol concentration, we can also exclude these factors as possible confounders.

We measured unbound VEGF levels in plasma. A decrease in free VEGF could in principle result from a concurrent upregulation of soluble VEGF receptor sFlt-1 (VEGF-R1). Vice versa, any change in the release of VEGF could become trapped by a concomitant counterregulation of the sFlt-1 receptor. In fact, the sFlt-1 receptor has been found to inhibit VEGF even more effectively than VEGF antibody (49). Moreover, previous investigations revealed an upregulation of Flt-1 (but not Flk-1/KDR) in response to hypoxia (9). On this background, we determined Flt-1 levels at baseline and at the time of the greatest decrease in VEGF concentration, i.e., at the end of the clamps. This measurement, however, did not indicate any differences between hypoxia and control in concentrations of unbound sFlt-1 and of the VEGF-sFlt-1 complex that could explain the decrease in free plasma VEGF upon hypoxia. In contrast, sFlt-1 showed a slight decrease in all three conditions, which was marginally significant during the hypoxic and control conditions. This offers a possible explanation for the apparent increase in VEGF during normoxic control, which was not significant. Considering the drop in sFlt-1 receptor concentration, the decrease of unbound VEGF after hypoxia appears even more pronounced. Why sFlt-1 decreased in all three conditions across the session independently of the experimental manipulations cannot be answered. Because the sessions always took place during the same time of day, the decrease might reflect effects of circadian rhythm, although to the best of our knowledge this has not been examined so far. However, in light of these data, the present...
failure of hypoxia to change expression of sFlt-1 appears to have some functional significance on its own, whereas concentrations of sFlt-1 remaining unaffected by hypoxia also argue against the view that the hypoxia-induced decrease in free VEGF represents increased binding of the molecule to its soluble receptor.

Our results show that VEGF concentrations increased over time during the normoxic control session. This increase may be explained by a circadian rhythm, which has been likewise found in normal mice during their active period of the day (21). Nevertheless, the question remains unresolved whether the administration of glucose or insulin affected the release of VEGF in our study. To clarify this issue, in a separate condition we reduced glucose infusion for 30 min while serum insulin was held stable. We found that VEGF concentrations increased during glucose reduction but were comparable to the normoglycemic control after a further 150 min. These data show that glucose concentration has an acute effect on plasma VEGF concentration, which, however, in contrast to the hypoxia induced decrease in VEGF, does not persist. We thus assume that the persisting increase in VEGF levels during the normoxic euglycemic control session occurring when both insulin and glucose are stable reflects mainly an effect of circadian rhythm, although we cannot exclude any interaction with other factors in intact organisms.

In contrast to previous in vitro studies, we found a decrease of VEGF in response to hypoxia. The explanation may possibly be linked to a confounder, which was discovered in another in vitro study: Lerman et al. (23) found that VEGF production in fibroblasts from diabetic, and therefore glucose-intolerant, mice was not upregulated in hypoxic conditions. Because it is known that hypoxia causes glucose intolerance in vivo (35), it may be concluded that VEGF production in our study was influenced by a glucose-intolerant state; but this would still not explain a decrease in plasma VEGF. However, our results also seem to be contradictory to some previous studies in patients with chronic hypoxic diseases. Notably, recent studies reported on increased serum/plasma levels of VEGF in patients with obstructive sleep apnea syndrome, a disease with recurrent hypoxic periods (15, 22, 43). However, these data still have to be carefully interpreted, also because the authors did not take into consideration that increased VEGF concentrations are likewise found in patients with metabolic comorbidities such as hypertension (1). The increased VEGF levels in patients with high blood pressure are reduced by the treatment of hypertension (1), as it occurs concomitantly by continuous positive airway pressure treatment in obstructive sleep apnea (26).

None of the named studies determined changes or even baseline values in blood pressure. Also, putative changes in VEGF receptor regulation were not investigated. Nevertheless, one possible role that the changes in circulating levels of VEGF may play is related to its enhancing influence on the expression of glucose transporter (GLUT)1 at the blood-brain barrier (BBB) (28, 45). Furthermore, VEGF mediates induction of endothelial fenestrations (4), thereby increasing transport of small molecules such as sucrose and fluorescein across the BBB (48, 50). Through these actions at the BBB, a decrease in VEGF as seen here after acute hypoxia could diminish glucose transfer across the BBB that eventually may be neuroprotective. But as our study did not determine the mechanism of the decrease, this hypothesis is presently highly speculative and needs ample research for confirmation. Aside from this issue, our data show that acute hypoxia decreases plasma VEGF levels in vivo at rest. This provides evidence that systemic VEGF concentration is differently regulated from the expression on a cellular basis, potentially underlying some factors not yet identified.
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REFERENCES


