Respiratory Medicine (2005) **99**, 1125–1131

respiratoryMEDICINE

Essential roles of angiotensin II in vascular endothelial growth factor expression in sleep apnea syndrome

Susumu Takahashi, Yutaka Nakamura*, Tsuguo Nishijima, Shigeru Sakurai, Hiroshi Inoue

Third Department of Internal Medicine, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka, Iwate, 020 8505, Japan

Received 6 November 2004

KEYWORDS
Obstructive sleep apnea syndrome;
Vascular endothelial growth factor;
Angiotensin II;
Angiotensin II receptor type 1;
Olmesartan

Summary
Background: Hypoxia-induced endothelial cell dysfunction has been implicated in increased cardiovascular disease associated with obstructive sleep apnea syndrome (OSAS). OSAS mediates hypertension by stimulating angiotensin II (Ang II) production. Hypoxia and Ang II are the major stimuli of vascular endothelial growth factor (VEGF), which is a potent angiogenic cytokine and also contributes to the atherogenic process itself.
Methods & results: We observed serum Ang II and VEGF levels and peripheral blood mononuclear cell (PBMC) and neutrophil VEGF expression. Compared to controls, subjects with OSAS had significantly increased levels of serum Ang II and VEGF and VEGF mRNA expression in their leukocytes. To examine whether Ang II stimulates VEGF expression in OSAS, we treated PBMCs obtained from control subjects with Ang II and with an Ang II receptor type 1 (AT_1) blocker, olmesartan. We observed an increased expression of VEGF in the Ang II-stimulated PBMCs and decreased in VEGF mRNA and protein expression in the PBMCs treated with olmesartan.
Conclusions: These findings suggest that the Ang II-AT_1 receptors pathway potentially are involved in OSAS and VEGF-induced vascularity and that endothelial dysfunction might be linked to this change in Ang II activity within leukocytes of OSAS patients.
© 2005 Elsevier Ltd. All rights reserved.

Introduction

Obstructive sleep apnea syndrome (OSAS) is associated with considerable cardiovascular morbidity

*Corresponding author. Tel.: +81 19 651 5111; fax: +81 19 626 8040.
E-mail address: icb75097@nifty.com (Y. Nakamura).

0954-6111/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2005.02.027

and mortality.[1,2] Several studies have identified OSAS as an independent risk factor for both nocturnal and diurnal hypertension.[3–5] Furthermore, the few published studies on 24 h blood pressure (BP) profiles in patients with OSA report disturbances of the circadian BP rhythm.[6,7] Møller et al. have shown that OSAS mediates hypertension by stimulating angiotensin II (Ang II) production.[8] Ang II is a multifunctional bioactive peptide. One important role of this peptide is regulation of blood pressure and blood flow by modification of vascular tone. Recently, many reports have suggested the significance of Ang II as a growth factor. Ang II has been shown to regulate growth of vascular smooth muscle cells,[9] induce local expression of endothelin-1 (ET-1) and vascular endothelial growth factor (VEGF).[10,11] These reports suggest that Ang II may play a role in the development of a particular role in vascular remodeling. VEGF is a dimorphic glycoprotein with a molecular weight of 34,000–42,000, consisting of two disulfide-linked peptide chains having identical N termini.[12] Four forms of VEGF have been described: $VEGF_{121}$ and $VEGF_{165}$, which are soluble products, and $VEGF_{189}$ and $VEGF_{206}$, which remain primarily cell associated and lack the mitogenic activity of the smaller forms.[13,14] This suggests that $VEGF_{165}$ and $VEGF_{121}$ are the pivotal forms of VEGF. VEGF has been identified as a cytokine that regulates differentiation, proliferation, migration, and survival of cells in the microvascular endothelium.[14,15] Along with a role in angiogenesis, recent studies have shown that VEGF may also contribute to the atherogenic process itself. VEGF-induced monocyte activation and migration[16] have been shown to modulate the growth of smooth muscle cells,[17] and be closely related to the progression of coronary atherosclerosis in humans[18,19] and the extent of carotid stenosis.[20] Several reports have shown that OSAS patients had elevated concentrations of VEGF that correlated with the severity of the OSAS as measured by the nocturnal hypoxia and apnea-hypopnea index (AHI).[21–23] It has also been reported that amelioration of the nocturnal hypoxia by nasal continuous positive air pressure (nCPAP) was associated with a significant decrease in morning VEGF concentrations.[24] In fact, hypoxia is the major stimulus that upregulates VEGF synthesis by controlling gene transcription and mRNA stabilization.[25,26] The involvement of Ang II in the pathogenesis of vascular dysfunction in sleep apnea, however, has not been investigated in detail. We cannot exclude the possibility that enhancement of VEGF expression is induced by hypoxia-induced Ang II stimulation.

Ang II may contribute to angiogenesis and vascular dysfunction by VEGF biosynthesis in patients with OSAS. In this study, we report that Ang II is a potent stimulator of VEGF in leukocytes through the Ang II receptor and that this Ang II-induced VEGF upregulation is suppressed by an Ang II receptor type 1 blocker, olmesartan.

Materials and methods

Patients and control subjects

The study was approved by the ethics committee of Iwate Medical University Hospital. The population included nine control subjects and 8 OSAS patients who participated in this study. All subjects were recruited from Iwate Medical University Hospital and provided written informed consent according to the ethical protocols of our institution. To be included in the study, patients had to be between 30 and 70 years of age, have a sleep laboratory finding of an AHI more than 5, and have no illness in the week preceding the study. Control subjects without apnea were recruited from patients who were referred for plysomnographic monitoring because of suspected sleep apnea but were found either to have primary snoring or an AHI score of less than 5, provided that most of the disordered breathing events were hypopneas not associated with arterial oxygen desaturations. All subjects were nonsmokers, asymptomatic and nonatopic with normal spirometry.

Measurement of Ang II and VEGF

Blood samples were drawn from subjects' antecubital veins at the end of each sleep study (i.e., 6 AM) and immediately centrifuged for 10 min at 3000 rpm. The supernatant was frozen at −20 °C for later analysis. Plasma Ang II was determined by radioimmunoassay, as previously described.[8] $VEGF_{165}$ serum and cultured supernatant levels were determined by a commercially available ELISA test (R&D System Inc., Minneapolis, MN). This assay is calibrated against a highly purified recombinant human $VEGF_{165}$, the major and most potent isoform of VEGF.

Leukocyte isolation

Peripheral blood mononuclear cell (*PBMC*): Heparinized venous blood was centrifuged on Histopaque 1077 (Sigma, Tokyo, Japan) at 1500 *G* for 15 min at room temperature. Cells were collected at the

interface and washed twice with Dulbecco's phosphate-buffered saline (Invitrogen Corp., Carlsbad, CA).

Neutrophils: Granulocyte/red blood cells (RBC) pellets were collected. The RBCs were removed by hypotonic lysis. Total neutrophil counts were calculated using a hemacytometer.

Cell culture

PBMC viability was determined by trypan blue exclusion. Cells were plated at a density of 2×10^6 cells/1 cm^2 growth area. Cells were resuspended in RPMI 1640 (Invitrogen Corp.) supplemented with 2 mM L-glutamine, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) pH 7.2, and incubated overnight at 37 °C, 5% CO_2 in air. PBMCs were stimulated with 100 nM Ang II. In experiments to examine the effects of inhibitors, the cultured cells were preincubated with olmesartan (RNH-6270, donated by Sankyo Pharmaceutical Co, Tokyo, Japan), a specific AT_1 receptor blocker, for 2 h before 100 nM Ang II treatment and then treated with Ang II concomitant with olmesartan (OL) for 24 h. Patient source (control vs OSAS) did not affect responsiveness to Ang II and OL.

RNA isolation and RT-PCR

RNA was extracted from isolated PBMCs, neutrophils, and cultured PBMCs by RNeasy (Qiagen, Valencia, CA) according to the procedure provided by the vendor. RT-PCR was performed as described previously.[27] To amplify the known splice variants, the following primer pairs that correspond to the 3′ and 5′ segments of the untranslated regions were used: VEGF forward primer, 5′-TCGGGCCTCCGAAA CCA TGA-3′; reverse, 5′-CCTGGTGAGAGATCTGGT TC-3′; Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward primer, 5′-TCGTGGAAGGACT-CATG-3′; reverse, 5′-AGTGTAGCCCAGGATGCC-3′. The amplified PCR product sizes of the $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$, and GAPDH RT-PCRs were 516bp, 648bp, 720bp, 771bp and 231bp, respectively. PCR without RT samples was performed as a negative control. The PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. Signals were quantified by densitometric scanning and normalized against GAPDH. Control group or 0 h cultured cells are standardized to 1.0.

Statistical analysis

All data are expressed as the mean ± SEM. The Tukey multiple comparison procedure was used to assess the statistical significance among the four groups. The student unpaired *t*-test was also used to examine changes between the control group and the OSAS patients after testing data for normality of the population using Kolmogorov–Smirnov test.

Results

Patient characteristics

Eight OSAS patients and eight control subjects completed the study. One patient from control group discontinued because of an upper respiratory tract infection. The clinical and demographic data of the two groups are summarized in Table 1. These data of the two groups demonstrated that patients with OSAS had a moderate to severe OSAS with a mean AHI of 54.6 ± 8.8 events/h. The two groups had no significant differences in age ($P = 0.64$), BMI ($P = 0.86$), the rate of hypertension, ischemic heart disease, and diabetes mellitus. There were significant differences in arousal index (ARI), average nightly SaO_2, and minimal SaO_2 ($P < 0.01$). Lung function parameters were within the normal range in all persons studied. Six hypertensive patients were well controlled at the outpatient clinic. They had a well-documented history of chronically elevated blood pressure (≥140/90 mm Hg) without any apparent underlying cause. All hypertensive patients took long-acting calcium channel blockers, and were not taking any other

Table 1 Demographic, sleep, and clinical data of control subjects and OSAS patients.

	Control ($n = 8$)	OSAS ($n = 8$)
Age (*y*)	55.8 ± 5.4	49.0 ± 3.9
Sex (male/female ratio)	8:0	8:0
BMI (kg/m^2)	28.0 ± 1.6	30.5 ± 1.2
AHI	2.3 ± 0.6	54.6 ± 8.8**
ARI (*n*/h TST)	13.3 ± 0.3	65.4 ± 9.1**
Average nightly SaO_2 (%)	95.0 ± 0.3	85.7 ± 2.3**
Minimal SaO_2 (%)	89.1 ± 0.9	59.8 ± 9.4**
Hypertension (*n*)	3	3
Ischemic heart disease (*n*)	0	0
Diabetes mellitus (*n*)	0	0

Values are expressed as mean ± SEM. BMI = body mass index; AHI = apnea-hypopnea index; ARI = arousal index; TST = total sleep time. ***P* < 0.01.

Table 2 Specific laboratory data for Ang II and VEGF.

	Control ($n=8$)	OSAS ($n=8$)
Plasma Ang II (pg/ml)	37.9±2.3	51.9±5.2*
(coefficient of variation)	(17.4)	(28.26)
Plasma VEGF (pg/ml)	300.8±35.3	502.1±63.7*
(coefficient of variation)	(33.24)	(35.86)

*$P<0.05$.

medication. Antihypertensive drugs were discontinued at least 5 days before the study.

Ang II and VEGF levels

The mean Ang II and VEGF plasma levels were markedly elevated in the OSAS group when compared to control group (Table 2).

VEGF mRNA expression in PBMC and neutrophils

As shown in Figs. 1A,B and 2A,B, PCR analysis of PBMCs as well as neutrophils detected VEGF mRNA in patients with OSAS and showed a slight expression in neutrophils.

ARB reduce Ang II-induced VEGF expression

To investigate the effect of Ang II on VEGF expression, PBMCs were stimulated with or without 100 nM Ang II. Ang II increased mRNA levels of VEGF by 2.2±0.1-fold in PBMCs. To determine whether an angiotensin II receptor blocker (ARB) effectively inhibited VEGF mRNA expression, the cultured PBMCs were preincubated with olmesartan for 2 h before 100 nM Ang II treatment and then treated with Ang II concomitant with olmesartan for 24 h. As shown in Fig. 3A and B, Ang II-induced VEGF mRNA expression was substantially reduced by olmesartan. Similar results in protein level were observed when supernatant of cultured PBMCs was subjected to ELISA (Fig. 4). The abundance of the VEGF protein decreased by 42% when pretreated with olmesartan.

Discussion

We have demonstrated that expression of both Ang II and VEGF is significantly increased in the plasma level of OSAS patients as compared to control

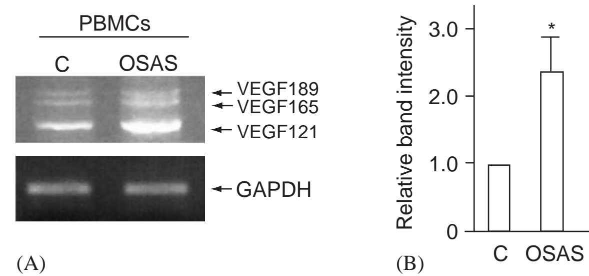

Figure 1 RT-PCR of VEGF mRNA on human leukocytes. Control (C) and obstructive sleep apnea syndrome (OSAS). VEGF mRNA expression was increased in peripheral blood mononuclear cell (PBMC) obtained from OSAS patients. A: Representative RT-PCR. Signals were quantified by densitometric scanning and normalized against Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Control groups are standardized to 1.0. The values in B are the mean±SEM of VEGF. *Indicates a statistically significant ($P<0.01$) difference from control group as determined by Student's *t*-test.

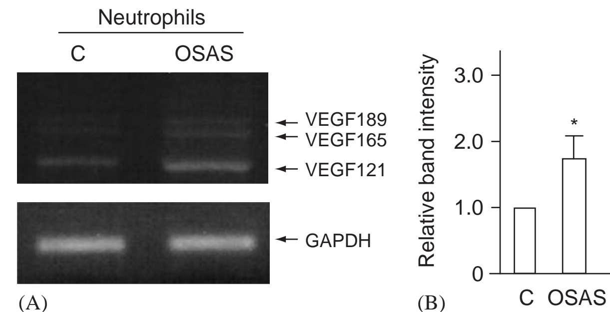

Figure 2 RT-PCR of VEGF mRNA on human leukocytes. Control (C) and obstructive sleep apnea syndrome (OSAS). VEGF mRNA expression was increased in neutrophils obtained from OSAS patients. A: Representative RT-PCR. Signals were quantified by densitometric scanning and normalized against Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Control group are standardized to 1.0. The values in B are the mean±SEM of VEGF. *Indicates a statistically significant ($P<0.01$) difference from control group as determined by Student's *t*-test.

subjects. In addition, we have shown that mononuclear cells and neutrophils expressed the VEGF in OSAS patients. Finally, we have shown that ARB olmesartan inhibited Ang II-induced enhancement of VEGF in vitro.

Prominent mechanisms in cardiovascular disease such as endothelial dysfunction (the earliest manifestation of atherosclerosis) were shown to be accentuated in patients with sleep apnea.[28,29] Two major effects of OSAS are currently under intense investigation: augmented sympathetic activation associated with sleep fragmentation and increased oxidative stress due to hypoxia/reoxygenation injury and possibly hypercapnia. Both may nega-

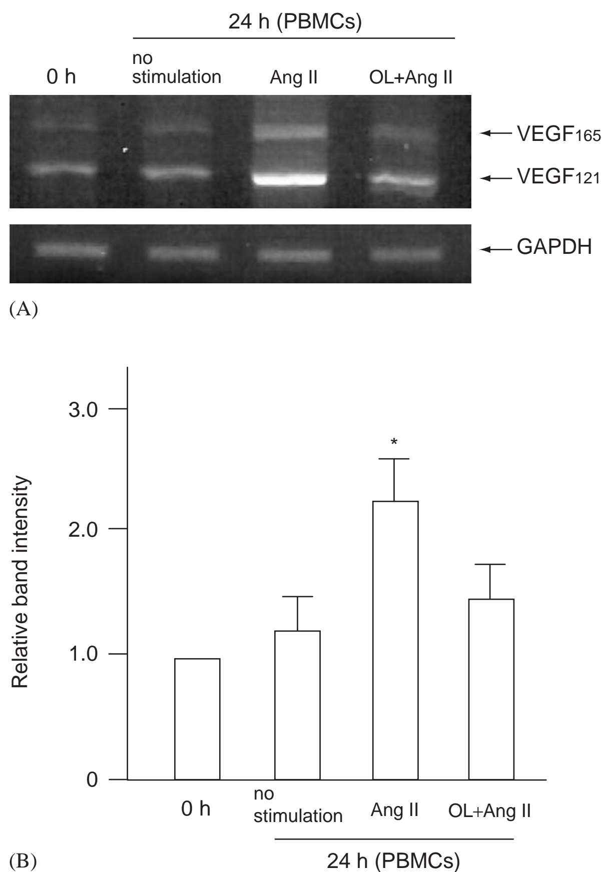

Figure 3 Effects of olmesartan on VEGF mRNA expression. Before the addition of 100 nM angiotensin II (Ang II), peripheral blood mononuclear cells (PBMCs) obtained from control subjects were pretreated with 100 nM olmesartan (OL). Total RNA was isolated from cultured PBMCs, and subjected to RT-PCR analysis. A: representative RT-PCRs. Signals were quantified by densitometric scanning and normalized against Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). 0 h cultured cells are standardize to 1.0. The values in B are the mean $\pm$ SEM of VEGF. *Indicates a statistically significant ($P<0.01$) difference from the other three groups as determined by the Tukey multiple comparison procedure.

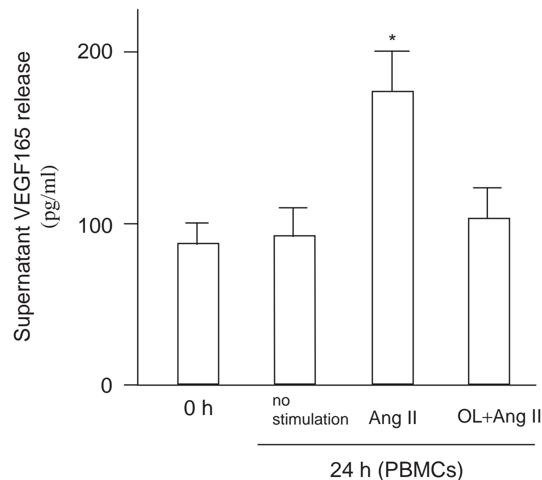

Figure 4 Levels of VEGF in peripheral blood mononuclear cells (PBMCs) culture supernatants. Before the addition of 100 nM Ang II, PBMCs were pretreated with the specific AT_1 receptor antagonist olmesartan 100 nM. Olmesartan (OL) diminishes Ang II-induced VEGF secretion from PBMCs. Results are expressed as mean $\pm$ SEM. *Indicates a statistically significant ($P<0.01$) difference from the other three groups as determined by the Tukey multiple comparison procedure.

tively affect cardiovascular function. In the present study we focused on the expression of VEGF among a variety of angiogenic factors because VEGF is induced by hypoxia. VEGF is expressed by a variety of cell types including aortic smooth muscle cells,[30] macrophages,[31] and myocytes.[32] In addition, lymphocytes,[33] neutrophils,[34,35] and platelets[36] have been shown to express VEGF. It was reported that PBMC- and neutrophil-derived VEGF contributed to vascular injury and remodeling in Kawasaki disease.[37] Furthermore, Mor et al. have recently demonstrated that T-cells play a role in angiogenesis by delivering VEGF to inflammatory sites, and that VEGF can augment proinflammatory T cell differentiation.[38] Our hypothesis is that expression of Ang II and VEGF is increased in the plasma level of individuals with OSAS patients. Using EIA, ELISA, and RT-PCR, we showed that Ang II and VEGF derived from leukocytes, especially PBMCs, were significantly increased in the OSAS patients compared with control subjects. It could be argued that the increased Ang II concentration in OSAS patients resulted from hypertension rather than from breathing disorders per se. However, this possibility seems as there were no significant differences in the rate of hypertension between the two groups. This will be further supported if significant reduction in plasma Ang II is demonstrated after successful nCPAP treatment. Given our in vivo data, we employed in vitro cultures of PBMCs obtained from control, subjects to evaluate the direct effect of Ang II on expression of VEGF. Our study has shown that the increase in VEGF expression was detected after stimulation of Ang II in PBMCs. As mentioned above, Ang II promotes several critical processes in atherosclerosis such as cell growth migration, release of growth factors, adhesion and chemoattractant molecules, and cytokines.[39] Ang II may induce in vascular cells the expression of the inflammatory cyclooxygenase (COX)-2 gene[40] and influence the extracellular matrix turnover by regulating the activity of prostaglandin E2-dependent metalloproteinase,[41] enzymes that degrade extracellular matrix and

thus weaken the fibrous cap of the atheromatous lesion, and promote its rupture.[42] Recently, Tazaki et al. demonstrated that serum MMP-9 is increased in OSAS patients when compared to normal subjects.[43] They speculated that elevated serum MMP-9 might induce vascular events in OSAS patients. Notably, all these effects appear mediated by Ang II type 1 (AT_1) receptors, as reflected by in vitro studies using selective Ang II receptor antagonists.[41] The biological effects of Ang II are mediated by Ang II receptors, of which two major mammalian subtypes, AT_1 and AT_2, have been identified.[44] The AT_1 receptor has been shown to promote angiogenesis and microvascular angiogenesis, which may be due to an AT_1 receptor-mediated upregulation of VEGF expression.[45] We pretreated cultured PBMCs with the selective AT_1 receptor blocker olmesartan to confirm the role of Ang II in regulating VEGF. VEGF expression decreased by 42%, suggesting that AT_1 is essential to VEGF expression after Ang II stimulation of PBMCs in vitro. However, local activation of VEGF-producing cells cannot explain the in vivo data in its entirety. To our knowledge, there have not been any clinical studies examining the effect of ARBs on VEGF expression in OSAS patients. Our in vitro data may be cautiously interpreted to support the concept of AT_1 receptor block for treatment of OSAS patients with ongoing vascular remodeling.

Apart from Ang II stimulation, the expression of the VEGF gene is stimulated by hypoxia through mediation of hypoxia-inducible factor (HIF).[46] HIF is also responsible for the induction of a variety of further hypoxia-sensitive genes. HIF-mediated gene expression might occur in OSAS patients as a result of repetitious nighttime hypoxia. In fact, our preliminary data demonstrated that HIF mRNA expression is increased in OSAS patients as compared to the control group. Moreover, PBMCs that were transfected with HIF antisense phosphorothioate oligonucleotides, reduced expression of VEGF mRNA (personal observation). However, we did not detect correlations between serum VEGF and AHI in our present study. It is possible that this study, which was limited due to the focus on patients with moderate to severe OSAS, was insufficient to observe such a correlation.

In conclusion, our study addresses the possibility that VEGF expression is induced, at least in part, by increased Ang II and that the potential therapeutic utility of ARBs as a novel approach for the treatment of patients with OSAS. Since vascular dysfunction is believed to be associated with enhanced risk for occlusive vascular disease in OSAS patients, the need for strategies aimed at modulating the Ang II function is compelling.

Acknowledgments

We thank our research subjects for participating in these studies. We acknowledge Mitsue Shibanai for assistance with subject recruitment and study procedures. The authors thank Sankyo Co., Ltd. for providing RNH-6270. We also thank Dr. Qutayba Hamid and Amy M. Neeno-Eckwall for their helpful discussion.

References

1. Koskenvuo M, Kaprio J, Partinen M, Langinvainio H, Sarna S, Heikkila K. Snoring as a risk factor for hypertension and angina pectoris. *Lancet* 1985;**ii**:893–6.
2. Koskenvuo M, Kaprio J, Telakivi T, Partinen M, Heikkila K, Sarna S. Snoring as a risk factor for ischaemic heart disease and stroke in men. *Br Med J* 1987;**294**:16–9.
3. Peppard PE, Young TB, Palta M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. *N Engl J Med* 2000;**342**:1378–84.
4. Nieto FJ, Young TB, Lind BK, et al. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. *JAMA* 2000;**283**:1829–36.
5. Grote L, Ploch T, Heitmann J, Knaack L, Penzel T, Peter JH. Sleep-related breathing disorder is an independent risk factor for systemic hypertension. *Am J Respir Crit Care Med* 1999;**160**:1875–82.
6. Lavie P, Yoffe N, Berger I, Peled R. The relationship between the severity of sleep apnea syndrome and 24-h blood pressure values in patients with obstructive sleep apnea. *Chest* 1993;**103**:717–21.
7. Pankow W, Nabe B, Lies A, et al. Influence of sleep apnea on 24-hour blood pressure. *Chest* 1997;**112**:1253–8.
8. Møller DS, Lind P, Strunge B, Pedersen EB. Abnormal vasoactive hormones and 24-hour blood pressure in obstructive sleep apnea. *Am J Hypertens* 2003;**16**:274–80.
9. Daemen MJ, Lombardi DM, Bosman FT, Schwartz SM. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. *Circ Res* 1991;**68**:450–6.
10. Imai T, Hirata Y, Emori T, Yanagisawa M, Masaki T, Marumo F. Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. *Hypertension* 1992;**19**:753–7.
11. Zhao Q, Ishibashi M, Hiasa K, Tan C, Takeshita A, Egashira K. Essential role of vascular endothelial growth factor in angiotensin II-induced vascular inflammation and remodeling. *Hypertension* 1994;**44**:264–70.
12. Senger DR, Connolly DT, Van de Water L, Feder J, Dvorak HF. Purification and NH2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. *Cancer Res* 1990;**50**:1774–8.
13. Breier G, Albrecht U, Sterrer S, Risau W. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. *Development* 1992;**114**:521–32.
14. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol Endocrinol* 1991;**5**:1806–14.
15. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/ vascular endothelial growth factor,

microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995;**146**:1029–39.
16. Clauss M, Gerlach M, Gerlach H, et al. Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. *J Exp Med* 1990;**172**:1535–45.
17. Doi K, Itoh H, Komatsu Y, et al. Vascular endothelial growth factor suppresses C-type natriuretic peptide secretion. *Hypertension* 1996;**27**(3 pt.2):811–5.
18. Inoue M, Itoh H, Ueda M, et al. Vascular endothelial growth factor (VEGF) expression in human coronary atherosclerotic lesions: possible pathophysiological significance of VEGF in progression of atherosclerosis. *Circulation* 1998;**98**:2108–16.
19. Clauss M, Weich H, Breier G, et al. The vascular endothelial growth factor receptor Flt-1 mediates biological activities. *J Biol Chem* 1996;**271**:17629–34.
20. Lee SW, Jeong MH, Bae HR, et al. Circulating levels of interleukin-8 and vascular endothelial growth factor in patients with carotid stenosis. *J Korean Med Sci* 2001;**16**:198–203.
21. Imagawa S, Yamaguchi Y, Higuchi M, et al. Levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea-hypopnea syndrome. *Blood* 2001;**98**:1255–7.
22. Schulz R, Hummel C, Heinemann S, Seeger W, Grimminger F. Serum levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea and severe nighttime hypoxia. *Am J Respir Crit Care Med* 2002;**165**:67–70.
23. Gozal D, Lipton AJ, Jones KL. Circulating vascular endothelial growth factor levels in patients with obstructive sleep apnea. *Sleep* 2002;**25**:59–65.
24. Lavie L, Kraiczi H, Hefetz A, et al. Plasma vascular endothelial growth factor in sleep apnea syndrome: effects of nasal continuous positive air pressure treatment. *Am J Respir Crit Care Med* 2002;**165**:1624–8.
25. Xiong M, Elson G, Legarda D, Leibovich SJ. Production of vascular endothelial growth factor by murine macrophages: regulation by hypoxia, lactate, and the inducible nitric oxide synthase pathway. *Am J Pathol* 1998;**153**:587–98.
26. Marti HH, Risau W. Systemic hypoxia changes the organ-specific distribution of vascular endothelial growth factor and its receptors. *Proc Natl Acad Sci USA* 1998;**95**:15809–14.
27. Nakamura Y, Esnault S, Maeda T, Kelly EAB, Malter JS, Jarjour NN. Ets-1 regulates TNF-α-induced matrix metalloproteinase-9 and tenascin expression in primary bronchial fibroblasts. *J Immunol* 2004;**172**:1945–52.
28. Kato M, Roberts-Thomson P, Phillips BG, et al. Impairment of endothelium- dependent vasodilation of resistance vessels in patients with obstructive sleep apnea. *Circulation* 2000;**102**:2607–10.
29. Kraiczi H, Caidahl K, Samuelsson A, Peker Y, Hedner J. Impairment of vascular endothelial function and left ventricular filling: association with the severity of apnea-induced hypoxemia during sleep. *Chest* 2001;**119**:1085–91.
30. Ferrara N, Winer J, Burton T. Aortic smooth muscle cells express and secrete vascular endothelial growth factor. *Growth Factors* 1991;**5**:141–8.
31. Fava RA, Olsen NJ, Spencer-Green G, et al. Vascular permeability factor/ endothelial growth factor (VPF/VEGF): accumulation and expression in human synovial fluids and rheumatoid synovial tissue. *J Exp Med* 1994;**180**:341–6.
32. Hashimoto E, Ogita T, Nakaoka T, Matsuoka R, Takao A, Kira Y. Rapid induction of vascular endothelial growth factor expression by transient ischemia in rat heart. *Am J Physiol* 1994;**267**:H1948–54.
33. Freeman MR, Schneck FX, Gagnon ML, et al. Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. *Cancer Res* 1995;**55**:4140–5.
34. Gaudry M, Bregerie O, Andrieu V, El Benna J, Pocidalo MA, Hakim J. Intracellular pool of vascular endothelial growth factor in human neutrophils. *Blood* 1997;**90**:4153–61.
35. Taichman NS, Young S, Cruchley AT, Taylor P, Paleolog E. Human neutrophils secrete vascular endothelial growth factor. *J Leukoc Biol* 1997;**62**:397–400.
36. Mohle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc Natl Acad Sci USA* 1997;**94**:663–8.
37. Hamamichi Y, Ichida F, Yu X, et al. Neutrophils and mononuclear cells express vascular endothelial growth factor in acute Kawasaki disease: its possible role in progression of coronary artery lesions. *Pediatr Res* 2001;**49**:74–80.
38. Mor F, Quintana FJ, Cohen IR. Angiogenesis-inflammation cross-talk: vascular endothelial growth factor is secreted by activated T cells and induces Th1 polarization. *J Immunol* 2004;**172**:4618–23.
39. Schmidt-Ott KM, Kagiyama S, Phillips MI. The multiple actions of angiotensin II in atherosclerosis. *Regul Pept* 2000;**93**:65–77.
40. Ohnaka K, Numaguchi K, Yamakawa T, Inagami T. Induction of cyclooxygenase-2 by angiotensin II in cultured rat vascular smooth muscle cells. *Hypertension* 2000;**35**:68–75.
41. Brilla CG, Zhou G, Rupp H, Maisch B, Weber KT. Role of angiotensin II and prostaglandin E2 in regulating cardiac fibroblast collagen turnover. *Am J Cardiol* 1995;**76**:8D–13D.
42. Cipollone F, Prontera C, Pini B, et al. Overexpression of functionally coupled cyclooxygenase-2 and prostaglandin E synthase in symptomatic atherosclerotic plaques as a basis of prostaglandin E(2)-dependent plaque instability. *Circulation* 2001;**104**:921–7.
43. Tazaki T, Minoguchi K, Yokoe T, et al. Increased levels and activity of matrix metalloproteinase-9 in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2004;**170**:1354–9.
44. Chiu AT, Herblin WF, McCall DE, et al. Identification of angiotensin II receptor subtypes. *Biochem Biophys Res Commun* 1989;**165**:196–203.
45. Tamarat R, Silvestre JS, Durie M, Levy BI. Angiotensin II angiogenic effect in vivo involves vascular endothelial growth factor-and inflammation-related pathways. *Lab Invest* 2002;**82**:747–56.
46. Semenza GL, Agani F, Feldser D, et al. Hypoxia, HIF-1, and the pathophysiology of common human diseases. *Adv Exp Med Biol* 2000;**475**:123–30.