Cellular and Molecular Biology [TM] **52**, N°8, 24-27
DOI 10.1170/T756

ISSN 1165-158X
2006 Cell. Mol. Biol.[TM]

MARINOBUFAGENIN IN HYPERTENSIVE PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

N.E. ZVARTAU,[1] A.O. CONRADY,[1] Y.V. SVIRYAEV,[1] O.P. ROTARI,[1] N.K. MERKULOVA,[1] A.L. KALINKIN,[2] E.V. SHLYAKHTO,[1] A.Y. BAGROV[3]✉

[1]Almazov Research Institute of Cardiology, Saint-Petersburg, Russia
[2]Center for Sleep Disorders, City Hospital nr. 83, Moscow Russia
[3]✉Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia Address for correspondence: Laboratory of Cardiovascular Science, NIA/NIH, 5600 Nathan Shock Drive, Baltimore, MD, 21224
Fax: +1(410) 558-8150; Email: BagrovA@mail.nih.gov

Received March 15th, 2006; Accepted April 3rd, 2006; Published May 15th, 2006

Abstract – Although obstructive sleep apnea (OSA) is an independent risk factor for hypertension, the underlying mechanisms are not clearly understood. Apnea and hypopnea episodes during sleep lead to sympathoactivation, decrease plasma pH, and predispose to sodium and volume retention. We hypothesized that, the latter could stimulate digitalis-like natriuretic/vasopressor hormones, endogenous ouabain (EO) and marinobufagenin (MBG). Overnight polysomnography (Embletta) and 24hrs blood pressure monitoring (SpaceLab 90207) was conducted in 52 consecutive patients with OSA (51±8 years; 40 males, 12 females) and in 48 age-matched hypertensive subjects without OSA. According to the polysomnography data, 17 patients had a mild degree of OSA (apnea/hypopnea index (AHI) 5-15), 17 patients-moderate (IAH 15-30) and 18 - severe OSA (IAH >30). Levels of MBG excretion co-varied with OSA severity (0.5±0.1, 0.9±0.04 and 1.2±0.06nmoles per 24hrs, respectively), while excretion of EO did not differ in patients with different degrees of OSA severity. Our observations suggest that MBG may be involved in the pathogenesis of hypertension in OSA, and may be a marker of OSA severity.

Key words: Obstructive sleep apnea, hypertension, Na/K-ATPase, marinobufagenin.

INTRODUCTION

Obstructive sleep apnea (OSA) is an independent risk factor for hypertension (19,22). One of the established mechanisms of blood pressure (BP) elevation in OSA is activation of sympathetic nervous system (26). The repetitive episodes of apnea/hypopnea during sleep are accompanied by hypoxia/hypercapnia, which results in sympathoactivation through reflex activation of chemoreceptors (15,18,26). Additionally, in patients with OSA, hypoxia/hypercapnia produces elevation in the end-tidal CO_2 ($PETCO_2$) (16). Heightened $PETCO_2$ leads to plasma acidification and activation of the Na-H exchanger (24,25). Accordingly, the activity of lymphocyte Na-H exchange was found to be increased in patients with OSA (28). Renal sodium retention and plasma volume expansion, which occur due to activation of renotubular Na-H exchange (1,2,11), are known to be the stimuli for endogenous digitalis-like Na/K-ATPase inhibitors, i.e., cardiotonic steroids (CTS) (5).

Abbreviations: **OSA**: obstructive sleep apnea; **BP**: blood pressure; **SBP**: systolic blood pressure; **DBP**: diastolic blood pressure; **CTS**: cardiotonic steroids; **MBG**: marinobufagenin; **EO**: endogenous ouabain; **$PETCO_2$**: partial pressure of CO_2; **AHI** - apnea/hypopnea index; **Na/K-ATPase**: sodium-potassium-activated adenosine triphosphatase; **SaO_2**: arterial hemoglobin O_2 saturation.

During plasma volume expansion, CTS become stimulated to promote natriuresis via inhibition of the Na/K-ATPase in renal tubules (9). The excessive CTS response results in the inhibition of the Na/K-ATPase in the vascular smooth muscle cells, activation of sodium-calcium exchange and vasoconstriction (8).

We hypothesized that in hypertensive patients with OSA, CTS may contribute to enhanced vascular tone, and investigated 24hrs renal excretion of two CTS, marinobufagenin (MBG) and endogenous ouabain (EO) and their relationships with the arterial pressure and other clinical parameters in patients OSA.

MATERIALS AND METHODS

Study population

The study population included 67 male and 33 female patients with at least 1 year history of arterial hypertension, aged 45 to 65 years (mean age 51.8±6.5 years), BMI more than 25kg/m^2. Exclusion criteria were as follows: secondary hypertension, chronic pulmonary diseases, cardiovascular diseases (ischemic heart disease, heart failure, arrhythmias, valve diseases), cerebral vascular diseases, neoplasy, any medication not allowing a washout time of two weeks, chronic concurrent diseases (renal, liver failure, diabetes, etc). All previously treated patients underwent 2 weeks of washout period. The study was approved by the local

Medical Ethics Committee, and written informed consent was obtained from all subjects before the study.

Experimental procedures

Subjects were examined in out-patient clinic. The standard examination included determination of weight and height, calculation of body-mass index, and office measurement of BP. Following examination, BP has been monitored for 24hrs. On the same day, the patients underwent an overnight sleep study, and 24hrs urines were collected. Next morning, after 12hrs of fasting venous blood samples were drawn for hormones measurement.

Blood pressure monitoring

Ambulatory 24hrs BP was measured by oscillometric method using a device «SpaceLabs 90027» («SpaceLabs medical», USA). The BP readings were obtained every 15min during the day (7 am to 11 pm) and every 30min during the night (11 pm to 7 am). The mean daytime and nighttime BP were calculated using real waking and sleeping times of each individual patient.

Sleep studies

Overnight polysomnography was performed using a portable recording device (Embletta, Australia). An episode of cessation of both nasal and oral airflow for a period of ≥10s has been defined as apnea. Hypopneas were defined as a decrease in arterial hemoglobin O_2 saturation (SaO_2) > 4% associated with a reduction in oronasal airflow to <50% of baseline. The apnea–hypopnea index (AHI) was calculated as follows: (total number of apneas + hypopneas)/(total sleep time in hours). OSA was diagnosed in patients with AHI ≥ 5 events per hour. According to AHI index, severity of OSA has been defined as mild (AHI = 5-15), moderate (AHI = 15-30), or severe (AHI > 30).

Imuunoassays

Urinary concentrations of MBG and EO were measured via competitive DELFIA fluoroimmunoassay base on polyclonal rabbit anti-MBG (serum aMBG-P) and rabbit anti-ouabain (serum aOU-SPB) antibodies, as reported previously in detail (13). Plasma insulin level was determined using AxSym Insulin kit (Abbott Diagnostics, USA). Plasma concentrations of leptin were measured using Active Human Leptin IRMA DSL-23100i (Diagnostic Systems Laboratories, Inc., USA).

The results were analyzed statistically using one-way ANOVA and Bonferroni test, two-tailed t-test, and linear regression analysis (GraphPad Prism 3 software).

RESULTS

The values for age, body mass index, and systolic and diastolic blood pressure for patients with OSA and hypertensive subjects from control group are presented in table 1. Anthropometrical data and values of office systolic BP (SBP) and diastolic BP (DBP) did not differ in both groups. There was no difference between average daytime SBP between patients with OSA and controls, while levels of average daytime DBP in patients with OSA were slightly, but significantly, higher than that in hypertensive subjects without OSA. The nighttime levels of both SBP and DBP in patients with OSA substantially exceeded those in the control group.

Table 1 Clinical data for patients with OSA and control group.

Variables	OSA patients (n=52)	Control group (n=48)	P
Apnea/hypopnea index	38.9±23.7	2.8±1.5	<0.001
Age (years)	54.1±7.4	53.6±7.4	>0.05
Weight (kg)	106.5±18.4	104.2±19.5	>0.05
Height (cm)	171.8±7.8	170.7±9.6	>0.05
BMI (kg/m²)	35.1±5.9	34.3±6.7	>0.05
Hypertension duration (years)	11.5±8.0	12.1±8.6	>0.05
Office SBP (mm Hg)	160±7.4	158±8.6	>0.05
Office DBP (mm Hg)	98±7.2	96±8.4	>0.05
Daytime SBP (mm Hg)	157±14.7	152±10.7	>0.05
Daytime DBP (mm Hg)	98±6.9	94±4.8	<0.05
Nighttime SBP (mm Hg)	160±14.6	138±10.9	<0.01
Nighttime DBP (mm Hg)	96±6.9	82±5.7	<0.001

Means±SD. Two-tailed t-test.

Levels of renal MBG excretion in patients with OSA were greater than those in hypertensive subjects without apnea (0.85 ± 0.07nmoles vs. 0.34 ± 0.05nmoles, P < 0.0001), and increased progressively with the severity of OSA (fig. 1).

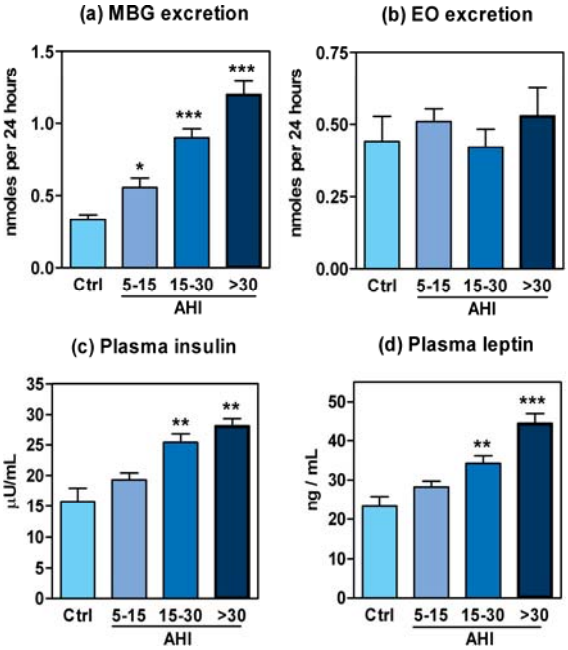

Fig. 1 Renal excretion of MBG (a) and EO (b), and plasma concentration of insulin (c) and leptin (d) in hypertensive patients from control group (Ctrl) and hypertensive patients with various degrees of OSA severity. AHI – apnea/hypopnea index Means ± SEM. By one-way ANOVA followed by Bonferroni test: * - P < 0.05; ** - P < 0.01, *** - P < 0.001 vs. Ctrl.

Unlike MBG, levels of EO excretion did not differ between OSA and control groups (0.51 ± 0.07nmoles vs. 0.39 ± 0.05nmoles), and did not vary in subgroups of patients with different OSA severity (fig. 1B).

As illustrated in fig. 1C and D, plasma concentrations of both insulin and leptin were elevated in patients with OSA as compared to control group. Similar to that of MBG, levels of insulin and leptin rose with the progression of OSA severity.

As presented in table 2, levels of 24hrs MBG excretion in patients with OSA exhibited a positive linear correlation with the severity of OSA, with levels of office, daytime, and nighttime DBP, and with plasma concentrations of insulin and leptin. None of these parameters exhibited significant correlations with levels of MBG excretion in the control group.

Table 2 Pearson correlations of 24hrs renal MBG excretion in hypertensive patients with and without OSA

Variables	MBG excretion in patients with OSA		MBG excretion in control group	
	r	P	r	P
AHI	0.70	<0.0001	0.23	0.10
Office SBP	0.46	0.06	0.24	0.70
Daytime SBP	0.37	0.06	0.28	0.60
Nighttime SBP	0.46	0.07	0.30	0.60
Office DBP	0.48	0.03	0.22	0.20
Daytime DBP	0.68	<0.01	0.22	0.10
Nighttime DBP	0.60	<0.01	0.20	0.20
Plasma leptin	0.44	0.03	0.30	0.10
Plasma insulin	0.37	0.04	0.28	0.10

DISCUSSION

The major observation of our study is that levels of MBG, but not EO, become elevated in hypertensive patients with OSA, and co-vary with the severity of OSA. This observation is in agreement with previous data obtained by Paci et al and demonstrating that hypertensive subjects with OSA exhibit elevated plasma levels of an unidentified endogenous Na/K-ATPase inhibitor, which, by its chromatographic behavior, is different from EO and resembles MBG (21). Furthermore, in another study, it has been observed that in healthy humans, the pressor response to voluntary hypoventilation is associated with a substantial inhibition of Na/K-ATPase in the erythrocytes, and with a simultaneous increase in plasma levels of marinobufagenin (5). Similar to that in the present study, levels of EO in these studies did not increase either in subjects with OSA (21), or during voluntary hypoventilation (5).

In NaCl-sensitive hypertension, MBG is stimulated with a primarily adaptive aim, of reducing the volume of circulating fluid, and to induce natriuresis via inhibition of renotubular Na/K-ATPase (4,9,13). An excessive MBG production also evokes a maladaptive response, i.e., inhibition of the sodium pump in the vascular smooth muscle membranes, which potentiates vasoconstriction (13). In OSA, repeated episodes of apnea/hypopnea lead to an increase in $PETCO_2$ (16). Previously both human and animal studies have demonstrated that heightened $PETCO_2$ levels decrease plasma pH, and stimulate renotubular Na-H exchange, which results in renal sodium retention, a major stimulus for MBG production (1,2,5,13). Thus, in patients with OSA, MBG may be an important link in the vicious circle underlying development of hypertension. In support of this notion, levels of MBG excretion in the present study positively correlated the severity of OSA, and with levels of DBP, while no such correlations were found in hypertensive subjects from control group.

In the present study, in patients with OSA, levels of MBG excretion co-varied with plasma levels of insulin and leptin. Insulin may contribute to renal sodium retention via stimulatory effect on the Na/K-ATPase, and via activation of renotubular epithelial sodium channels (27). We hypothesize that, at least in part, enhanced production of MBG observed in the present study might have occurred in an attempt to override sodium retention caused by insulin. OSA is associated with impaired glucose tolerance (17,23), and dysregulation of the Na/K-ATPase has been shown to contribute to the pathogenesis of diabetes mellitus (20,27). Recently, we have demonstrated that in diabetic patients and in rats with streptozotocin-induced with type 1 and type 2 diabetes mellitus, levels of MBG increase and contribute to Na/K-ATPase inhibition (6,7). In healthy rats, in vivo blockade of MBG with a specific antibody impaired glucose tolerance during oral glucose challenge (7). We hypothesize that MBG may be one of the factors linking mechanisms of pathogenesis of hypertension and insulin resistance in patients with OSA.

MBG is a potent vasoconstrictor and at physiologically relevant concentrations it induces Na/K-ATPase inhibition and elicits contractile responses in isolated human arteries (3,10). Since

enhanced MBG production in OSA is likely to be one of the factors contributing to enhanced vascular tone, pharmacological antagonism of the prohypertensive effects of this hormone may be one of the approaches to the treatment of hypertension patients with OSA. The strategies for therapeutic intervention against MBG and other CTS include disrupting the interaction of CTS and vascular sodium pump either via blockade of digitalis receptor on the sodium pump (12) or, by reduction of CTS sensitivity of the Na/K-ATPase via modulation of its protein kinases-induced phosphorylation (14).

Acknowledgment – This paper is dedicated to the memory of Dr. Anna Paci, whose research prompted our study. Authors gratefully acknowledge critical comments of Drs. David E. Anderson and Olga V. Fedorova, National Institute on Aging, NIH, Baltimore, MD.

REFERENCES

1. Anderson, D.E. Cardiorenal effects of behavioral inhibition of breathing. Biol. Psychol. 1998, **49**: 151-163.
2. Anderson, D.E., Bagrov, A.Y., and Austin, J.L. Inhibited breathing decreases renal sodium excretion. Psychosom. Med. 1995, **57**: 373-380.
3. Bagrov, A.Y., Dmitrieva, R.I., Fedorova, O.V., Kazakov, G.P., Roukoyatkina, N.I., and Shpen, V.M. Endogenous marinobufagenin-like immunoreactive substance. A possible endogenous Na, K-ATPase inhibitor with vasoconstrictor activity. Am. J. Hypertens. 1996, **9**: 982-990.
4. Bagrov, A.Y., and Fedorova, O.V. Cardenolide and bufadienolide ligands of the sodium pump. How they work together in NaCl sensitive hypertension. Front. Biosci. 2005, **10**: 2250-2256.
5. Bagrov, A.Y., Fedorova, O.V., Austin-Lane, J.L., Dmitrieva, R.I., and Anderson, D.E. Endogenous marinobufagenin-like immunoreactive factor and Na,K-ATPase inhibition during voluntary hypoventilation. Hypertension. 1995; **26**, 781-788.
6. Bagrov, Y.Y., Manusova, N.B., Egorova, I.A., Fedorova, O.V., and Bagrov, A.Y. Endogenous digitalis-like ligands and Na/K-ATPase inhibition in experimental diabetes mellitus. Front. Biosci. 2005, **10**: 2257-2262.
7. Bagrov, Y.Y., Manusova, N.B., Egorova, I.A., Frolova, E.V., Fedorova, O.V., and Bagrov, A.Y. Marinobufagenin, an endogenous inhibitor of alpha-1 Na/K-ATPase, is a novel factor in pathogenesis of diabetes mellitus. Dokl. Biol. Sci. **2005**, 404: 333-337.
8. Blaustein, M.P. Physiological effects of endogenous ouabain: control of intracellular Ca^{2+} stores and cell responsiveness. Am. J. Physiol. 1993, **264**: C1367-C1387.
9. Buckalew, V.M. Endogenous digitalis-like factors. An historical overview. Front Biosci. 2005, **10**: 2325-2334.
10. Fedorova, O.V., Dorofeeva, N.A., Lopatin, D.A., Lakatta, E.G., and Bagrov, A.Y. Phorbol diacetate potentiates Na-K-ATPase inhibition by a putative endogenous ligand, marinobufagenin. Hypertension. 2002, **39**: 298-302.
11. Fedorova, O.V., French, A.W., and Anderson, D.E. Inhibition of erythrocyte Na,K-ATPase activity during anticipatory hypoventilation in micropigs. Am J Hypertens. 1996, **9**: 1126-1131.
12. Fedorova, O.V., Talan, M.I., Agalakova, N.I., Droy-Lefaix, M.T., Lakatta, E.G., and Bagrov, A.Y. Myocardial PKC beta2 and the sensitivity of Na/K-ATPase to marinobufagenin are reduced by cicletanine in Dahl hypertension. Hypertension. 2003, **41**: 505-511.
13. Fedorova, O.V., Talan, M.I., Agalakova, N.I., Lakatta, E.G., and Bagrov, A.Y. Endogenous ligand of alpha(1) sodium pump, marinobufagenin, is a novel mediator of sodium chloride-dependent hypertension. Circulation. 2002, **105**: 1122-1127.
14. Ferrari, P., Ferrandi, M., Valentini, G., and Bianchi, G. Rostafuroxin: an ouabain antagonist that corrects renal and vascular Na+K+ ATPase alterations in ouabain and adducin-dependent hypertension. Am. J. Physiol. 2006, **290**: R529-R535.
15. Fletcher, E.C. Cardiovascular consequences of obstructive sleep apnea: experimental hypoxia and sympathetic activity. Sleep, 2000, **23**(Suppl 4): S127-S131.
16. Fuse, K., Satoh, M., Yokota, T., Ohdaira, T., Muramatsu, Y., Suzuki, E., and Arakawa, M. Regulation of ventilation before and after sleep in patients with obstructive sleep apnoea. Respirology. 1999, **4**: 125-130.
17. Levinson, P.D., McGarvey, S.T., Carlisle, C.C., Eveloff, S.E., Herbert, P.N., and Millman, R.P. Adiposity and cardiovascular risk factors in men with obstructive sleep apnea. Chest. 1993, **103**: 1336-1342.
18. Narkiewicz, K., and Somers, V.K. The sympathetic nervous system and obstructive sleep apnea: implications for hypertension. J. Hypertens. 1997, **12**: 1613-1619.
19. Nieto, F.J., Young, T.B., Lind, B.K., Shahar, E., Samet, J.M., Redline, S., D'Agostino, R.B., Newman, A.B., Lebowitz, M.D., and Pickering, T.G. Association of sleep-disordered breathing, sleep apnea, and hypertension in a large community-based study. Sleep Heart Health Study. JAMA. 2000, **283**: 1829-1836.
20. Ng, Y.C., Tolerico, P.H., and Book C.B. Alterations in levels of Na(+)-K(+)-ATPase isoforms in heart, skeletal muscle, and kidney of diabetic rats. Am. J. Physiol. 1993, **265**, E243-E251.
21. Paci, A., Marrone, O., Lenzi, S., Prontera, C., Nicolini, G., Ciabatti, G., Ghione, S., and Bonsignore, G. Endogenous digitalislike factors in obstructive sleep apnea. Hypertens. Res. 2000, **23**(Suppl): S87-S91.
22. Peppard, P.E., Young, T., Palta, M., and Skatrud, J. Prospective study of the association between sleep-disordered breathing and hypertension. N. Engl. J. Med. 2000, **342**: 1378-1384.
23. Punjabi, N.M., and Polotsky V.Y. Disorders of glucose metabolism in sleep apnea. J. Appl. Physiol. 2005, **99**: 1998-2007.
24. Quednau, B., Rosskopf, D., Reusch, H.P., Luft, F.C., and Siffert, W. Enhanced Na(+)-H+ exchanger activity and NHE-1 mRNA levels in human lymphocytes during metabolic acidosis. Am. J. Physiol. 1994, **266**: C480-C488.
25. Rios, E.J., Fallon, M., Wang, J., and Shimoda, L.A. Chronic hypoxia elevates intracellular pH and activates Na+/H+ exchange in pulmonary arterial smooth muscle cells. Am. J. Physiol. 2005, **289**: L867-L874.
26. Somers, V.K., Dyken, M.E., Clary, M.P., and Abboud, F.M. Sympathetic neural mechanisms in obstructive sleep apnea. J. Clin. Invest. 1995, **96**: 1897-1904.
27. Sweeny, G., and Klip, A. Regulation of the Na/K ATPase by insulin: why and how? Mol. Cell. Biochem. 1998, **182**: 121-133.
28. Tepel, M., Sanner, B.M., van der Giet, M., and Zidek, W. Increased sodium-proton antiporter activity in patients with obstructive sleep apnoea. J. Sleep. Res. 2000, **9**: 285-291.