Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 366 (2008) 180–185
BBRC
www.elsevier.com/locate/ybbrc

Adenosine A_{2B} receptor mediates an increase on VEGF-A production in rat kidney glomeruli

D. Valladares [a], C. Quezada [a], P. Montecinos [a], Concha II [a], A.J. Yañez [a], L. Sobrevia [b], R. San Martín [a,*]

[a] *Laboratorio de Patología Molecular (LPM), Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, P.O. Box 567, Campus Isla Teja, Valdivia, Chile*

[b] *Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics and Gynecology, Medical Research Centre (CIM), School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile*

Received 15 November 2007
Available online 3 December 2007

Abstract

Up-regulation of the glomerular expression and the activity of vascular endothelial growth factor-A (VEGF) have been identified as an early pathogenic event for the progression of diabetic nephropathy. Currently, however the mediators are not yet clearly recognized. In this study we identified all four adenosine receptor (AR) subtypes, i.e. A_1, A_{2A}, A_{2B} and A_3 in isolated rat kidney glomeruli. We localized the expression of A_{2B}AR in podocytes, the primary VEGF producing cells. The ex vivo treatment of kidney glomeruli with adenosine or a general AR agonist NECA, increases VEGF protein content. In addition, NECA treatment elicits VEGF release. These effects were blocked by the A_{2B}AR selective antagonist MRS1754 supplementation. Furthermore, we showed that A_{2B}AR activation was necessary to promote a higher expression of VEGF in kidney glomeruli upon exposure to high D-glucose concentration, a pathogenic condition like those observed in diabetic nephropathy.

Keywords: VEGF-A; Adenosine receptor; Glomerulus; High D-glucose

The vascular endothelial growth factor-A (VEGF-A or VEGF) is a homodimeric glycoprotein of 45 kDa with potent effects on vascular permeability and angiogenesis [1]. It is also important for normal nephrogenesis to occur [2], while the adult kidney maintains an unusual constitutive expression of VEGF mainly restringed to glomerular visceral epithelial cells (podocytes), renal tubule cells and collecting ducts [3]. Relevant glomerular functions are dependent on VEGF activity [4]. Among the proposed roles are the regulations of blood flow through the capillary tuft and to maintain the filtration activity [5]. Autocrine VEGF production could play a role in the homeostasis and podocytes survival [6] and in the expression of the filtration barrier protein nephrin [7]. It has also been implied in mesangial cell survival and differentiation [8].

The increase in glomerular VEGF expression and activity observed at early stages of the human diabetic nephropathy and in experimental models of diabetes is the consequence of the pathological role of this growth factor [3]. In addition, the renal VEGF over production by transgenesis demonstrated that it directly causes the glomerular hypertrophy that is associated with proteinuria [9].

The most potent stimuli for VEGF production is hypoxia [10] and also hyperglycemia [11]. Particularly, it has been demonstrated that in a mouse podocytes cell line the expression of VEGF increases under exposition to high D-glucose concentrations [12,13]. At present however, it is not clear how glomerular VEGF production is up-regulated in response to diabetes or high glucose concentration.

The nucleoside adenosine regulates essential renal functions by means of local modification of its extracellular

* Corresponding author. Fax: +56 63 221106.
E-mail address: rodysanmartin@uach.cl (R. San Martín).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.11.113

bioavailability to activate members of the P_1 family of purinoceptors that contain A_1, A_{2A}, A_{2B} and A_3 adenosine receptor (AR) subtypes [14]. However, very little is known about adenosine receptor expression and function in glomerular cells types.

Materials and methods

Glomeruli isolation. Renal cortex of male rats (Sprague–Dawley) weighing 200–250 g was sieved through 212 μm, 150 μm, 106 μm and 75 μm meshes. The material collected with the narrowest sieve corresponded to glomeruli [15].

Experimental treatment conditions. Purified glomeruli (10,000 per well) were incubated in 2 ml of HAM-F10 medium (5 mM D-glucose) (Invitrogen, USA) supplemented with 10 μmol/L adenosine, 1 μmol/L NECA (non selective P_1 receptors agonist), 50 nmol/L MRS1754 (A_{2B}AR antagonist), 100 nmol/L CGS21680 (A_{2A}AR agonist), 10 nmol/L ZM241385 (A_{2A}AR antagonist), 30 nmol/L CPA (A_1AR agonist), 30 nmol/L DPCPX (A_1AR antagonist) or D-glucose 25 mM at standard conditions (37 °C and 5% CO_2) for 6 h. Following incubation, the glomeruli were collected by centrifugation and supernatants were stored at −70 °C. Total protein extracts were obtained from glomeruli resuspended in 150 μl RIPA buffer containing protease inhibitors (1 mM PMSF, 2 μM aprotinin, 1 μg/μl leupeptin and pepstatin). The concentrations of adenosine receptors modulators were derived from Fredholm et al. [16].

Western blots. Total proteins extracts (100 μg) fractionated under none reducing conditions by polyacrylamide gel (10%) electrophoresis were transferred to nitrocellulose membranes and probed with monoclonal anti-VEGF antibody (1:1000) (C-1, Santa Cruz Biotechnology, USA). Membranes were washed in Tris buffer saline 0.1% Tween, and incubated (1 h) in TBST/0.1% BSA containing HRP-conjugated goat anti-mouse IgG antibody. Immunodetections were revealed by enhanced chemiluminescence and quantitated by densitometry. Following the stripping procedure the membranes were probed with a monoclonal anti-β actin antibody (1:5000) (Sigma–Aldrich, USA) and revealed as described above [17].

Reverse transcription. Total RNA was isolated from glomeruli using the Trizol® Reagent (Invitrogen, USA) [18]. RNA quality and integrity were assured by gel visualization and spectrophotometric analysis ($OD_{260/280}$), quantified at 260 nm and precipitated to obtain 2 μg/μl. Aliquots of 1 μg of total RNA were reversed transcribed into cDNA using oligo (dT_{18}) plus random hexamers (10-mers) and MMLV reverse transcriptase (Invitrogen, USA) [19].

Polymerase chain reaction. PCR were performed in a total volume of 20 μl containing 1 μl of cDNA (dilution 1:10), 1× PCR buffer, 1.5 mM Mg^{+2}, 0.4 mM dNTP's, 2U Taq DNA polymerase (Invitrogen, USA) and 0.5 μM of gene-specific oligonucleotide primers. Samples were incubated for 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 56 °C, 30 s at 72 °C and a final extension of 5 min at 72 °C. RTPCR products were sequenced in both directions by Taq dideoxyterminator cycle sequencing with the automated ABI Prism 3730 DNA sequencer (Applied Biosystems/Hitachi). Rat specifics oligonucleotide primers were: A_1AR 5′-CTCCATTCTGGCTCT GCTCG-3′ and 5′-ACACTGCCGTTGGCTCTCCA-3′, A_{2A}AR 5′-C CA TCTTTAGCCTCTTGGCT-3′ and 5′-AATCCGTAGGTAGATGGC CA-3′, A_{2B}AR 5′-TTCTGCACGGACTTTCACAG-3′ and 5′-AAGG AGTCAGTCCAATGCCA-3′, A_3AR 5′-TGGAGGTCCAGATGCACT TC-3' and 5′-CGAAACGGAAGTGGCATGAG-3′, β actin 5′-GATGA CCCAGATCATGTTTG-3′ and 5′-CAGGAGGAGCAATGATCTTG-3′.

Immunohistochemistry. Rat kidney tissues were fixed in formalin, paraffin embedded and 5 μm sections were mounted on xylanized slides. For immunodetection the slides were sequentially deparaffined and rehydrated, incubated with 10 mM sodium citrate (pH 6.0) for 30 min, hydrogen peroxide (70% methanol, 3% perhydrol) for 5 min, and blocked with PBS 1× containing 1% bovine serum albumin, 0.3% Triton X-100 and 5% fat free milk for 30 min at room temperature. The slides were incubated with polyclonal anti-A_{2B}AR (1:1000) antibody (R-20, Santa Cruz Biotechnology, USA) in blocking solution over night at 4 °C. Then, the slides were washed three times with PBS 1× for 5 min and the immunosignals revealed using the LSAB+System-HRP system (Dako-Cytomation, USA) [18].

Enzyme-linked immunosorbent assay (ELISA). The amounts of VEGF secreted by glomeruli were measured by a quantitative solid-phase ELISA enzyme immunoassay designed to recognize rat $VEGF_{164}$ (R&D Systems, USA). The sensitivity of the assay was 8.4 pg/ml.

Statistical analysis. Values are means ± SEM, where n indicates number of animals. Statistical analyses were carried out on raw data using the Peritz F multiple means comparison test. Student's t-test was applied for unpaired data.

Results

Adenosine receptor subtypes in rat kidney glomeruli

We obtained reproducible glomeruli preparations with a high degree of purity with less than 5% of renal tubules contamination. Glomeruli mostly lack of bowman capsule thus, allowing the ex vivo exposure of the glomerular cells to incubation media, as desired.

While in isolated rat kidney glomeruli we identified the expression of all four AR genes family, only A_1AR and A_{2B}AR transcripts were present in cultured podocytes (Fig. 1A). Using immunohistochemistry in rat kidney sections we recognized the expression of A_{2B}AR mainly in glomerular podocytes localized toward bowman's space and wrapping the capillary tuft (Fig. 1B).

Effect of the adenosine receptors activation on VEGF-A production

The VEGF production in adult rat kidney glomeruli has been mainly recognized in podocytes therefore, we evaluated the effect of activating the AR present in this cell type on VEGF expression. First we established that the activation of adenosine receptors using the endogen ligand adenosine (10 μM) after 6 h of stimulation increased the active VEGF dimeric 45 kDa protein content over basal levels in 66 ± 16.8% ($P < 0.01$). At 15 h the VEGF content decreased nearly to the level observed at time 0 (Fig. 2A). Furthermore, we exposed the glomeruli to the general AR agonist NECA. Similarly, NECA (1 μM) induced an increase of the VEGF expression (36 ± 7.1%, $P < 0.01$) (Fig. 2B).

The increase of glomerular VEGF induced by NECA was blocked by MRS1754, an A_{2B}AR subtype antagonist (Fig. 3A). On the other hand, neither addition of A_1AR subtype agonist (CPA) nor A_{2A}AR agonist (CGS21680) were able to increase VEGF content in glomeruli (Fig. 3A).

Furthermore, we looked for a correlation between the increased expression of VEGF upon A_{2B}AR subtype activation and VEGF release. The general P_1 agonist NECA (1 μM) increased the VEGF content in the incubation medium more than 4-fold over the constitutive amount released from rat kidney glomeruli (23 ± 0.7 versus 119 ± 0.7 ng/ml, $P < 0.01$). This augmented VEGF

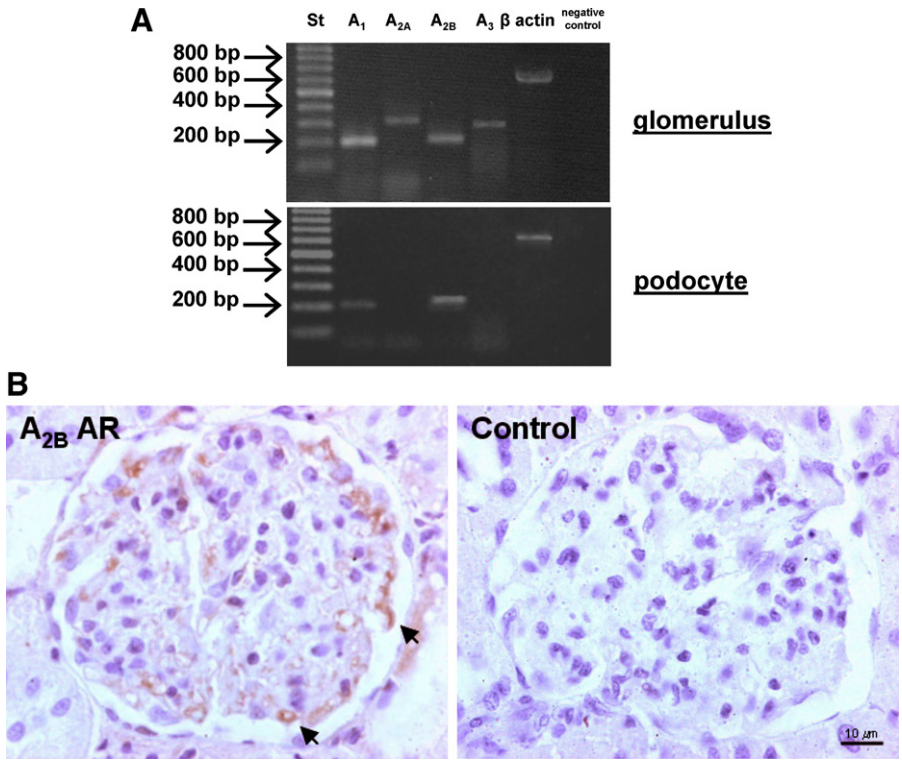

Fig. 1. Adenosine receptor subtypes expression in rat kidney glomeruli. (A) RT-PCR products of adenosine receptor subtypes transcript amplifications from rat glomeruli and podocytes (see Supplementary material). Expected amplification products were: A_1AR, 207 bp; A_{2A}AR, 320 bp; A_{2B}AR, 211 bp; A_3AR, 291 bp; β actin, 647 bp. (B) Immunohistochemical localization of A_{2B}AR expression in glomerular cells. Immunodetection was observed mainly in glomerular podocytes (arrows, left panel).

release induced by NECA did not occur in the presence of the A_{2B}AR antagonist MRS1754 (Fig. 3B).

Role of the A_{2B}AR subtype on increased VEGF-A expression upon exposure of glomeruli to high D-glucose concentration

A well recognized stimulus increasing VEGF expression in glomerular podocytes is the exposure to high glucose levels therefore, glomeruli were exposed to 5 mM (control) and 25 mM (high glucose) D-glucose concentration. High glucose induced a 2.9-fold increase ($P < 0.01$) in VEGF expression over constitutive VEGF protein content at 5 mM (Fig. 4A). Similarly, the expression of A_{2B}AR was up-regulated follows high glucose treatment (Fig. 4B and C). No variations on VEGF contents were evident when incubation media were supplemented with mannitol (data not shown). The effect of high glucose on the VEGF protein content was completely blocked when the co-treatment with the A_{2B}AR antagonist MRS1754 was performed (Fig. 4A).

Discussion

Only few studies have identified AR in glomerular cells types, but they lack to recognize a physiological function in this compartment [20–22]. VEGF expression in renal glomeruli has shown to be constitutive and podocytes are the main cell type involved in its normal and pathological production [4]. This is the first study that correlates the localization of A_{2B}AR, mainly found in rat glomerular podocytes, and a functional role on VEGF production. Previously, it was shown that the exposure of podocytes to high glucose concentration was an effective stimulus to induce VEGF expression, a process that requires the involvement of PKC and ERK signal pathways [12]. We observed an increase on VEGF expression following ex vivo incubation of rat glomeruli under high glucose concentrations, and interestingly this effect was completely dependent on adenosine A_{2B} receptor activity (Fig. 4A). This can be of great interest in pathologies where misregulations of plasma glucose concentrations occur, like diabetes mellitus or insulin resistance. It has been shown that the expression of VEGF increases in podocytes from diabetic patients in the early stages of diabetic kidney disease. In addition, the urinary excretion of VEGF increases according to the degree of proteinuria in both humans and diabetic rats [23,24]. It would be of great relevance to evaluate whether A_{2B} receptor antagonists would inhibit the progression of diabetes-associated nephropathy by a mechanism capable of decreasing VEGF over production by renal podocytes.

The glucose induced up-regulation of VEGF in renal glomeruli would require increases in extracellular adenosine bioavailability in order to activate low affinity A_{2B} receptors ($K_d > 1$ μM) [16]. It was reported previously that the extracellular adenosine content increased

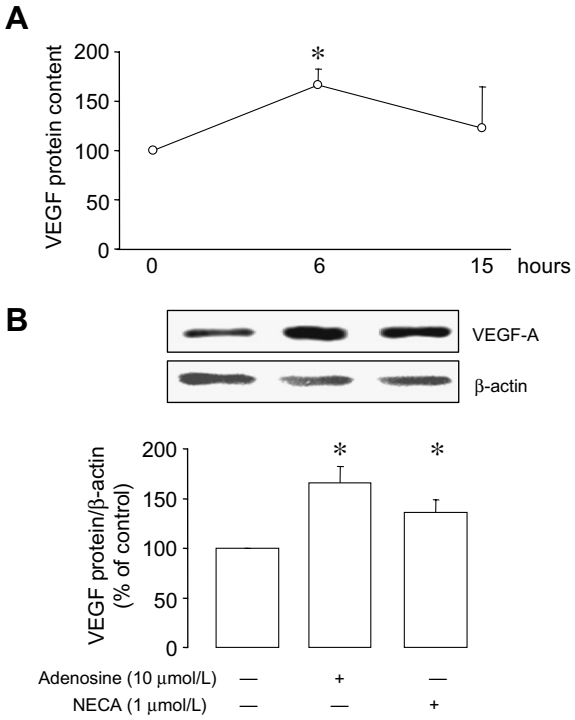

Fig. 2. Effects of general adenosine receptor agonist on VEGF-A expression in rat glomeruli. (A) Dimeric VEGF-A protein content in glomeruli at 0, 6 or 15 h exposure to 10 μM adenosine. The VEGF content variations were relative to β actin protein abundance in total protein extracts. $^{*}P < 0.01$ versus time 0 h ($n = 7$). (B) Western blot (representative) for VEGF-A and β actin in total protein extracts (100 μg) from glomeruli incubated for 6 h in absence (control) or presence of 10 μM adenosine or 1 μM NECA. $^{*}P < 0.01$ versus control value. The graph depicts the VEGF/β actin ratio immunosignals densitometry. The VEGF/β actin ratio in $t = 0$ or in control were normalized to 100%. Mean ± SEM ($n = 8$).

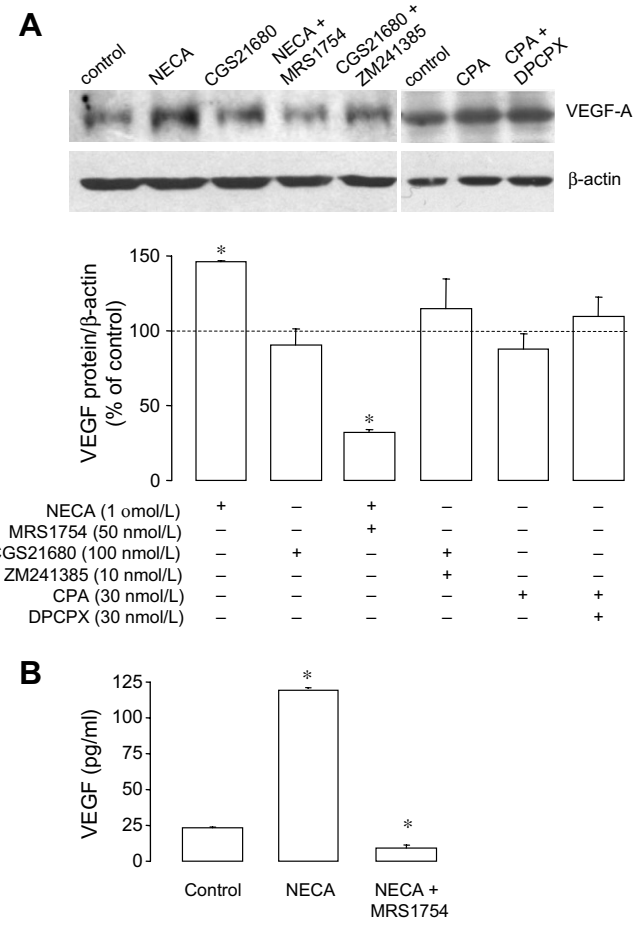

Fig. 3. Effects of adenosine receptors pharmacological modulators on glomerular VEGF-A production. (A) Rat glomeruli were exposed for 6 h to AR agonists and antagonists (see Materials and methods). Representative Western blot of dimeric VEGF and β actin in total protein extracts (100 μg) from glomeruli are shown. Control indicates absence of supplementation with pharmacological modulators. The graph shows the VEGF/β actin ratio immunosignals on Western blots. The VEGF/β actin ratio in control was normalized to 100%. $^{*}P < 0.01$ versus control value. Mean ± SEM ($n = 9$). (B) VEGF release to incubation medium. Rat glomeruli (10,000 per well) were incubated by 6 h in the absence (control) or presence of 1 μM NECA (general AR agonist) or 1 μM NECA in combination with 50 nM MRS1754 (selective A_{2B}AR antagonist). The graphs show the means ± SEM of VEGF content values in the supernatants. $^{*}P < 0.01$ versus control value ($n = 5$).

over 1 μmolar in human endothelial cells (HUVEC) isolated from gestational diabetes patients [19] and cultured under high glucose conditions [25] or hypoxia [17]. These increments are explained by the cell lower capacity for adenosine uptake under these conditions leading to the outer accumulation of this nucleoside. This effect has also been observed in lymphocytes from diabetic rats or in those exposed to high glucose concentrations [26]. In the kidney, a rise of extracellular adenosine bioavailability occurs to mediate an afferent arteriole resistance increase in order to respond to the tubuloglomerular feedback [27]. In addition, systemic hypoxic conditions rise renal cortex interstitial adenosine level to near a micromolar range [28]. Despite de fact that an adenosine depletion in afferent arterioles has been linked to renal hyperfiltration in the early diabetic kidney [14], the glomerular adenosine local content in normal and pathological conditions have yet to be assessed. However, the predominant expression of the 5′ ectonucletidase (CD73) (AMP→adenosine activity) is a remarkable feature in this renal compartment [27].

The effect upon activation of A_{2B} receptor in ex vivo glomerulus increased more than 4-fold the basal levels of VEGF release (Fig. 3B). Similar findings were reported on angiotensin II stimulation of a mouse podocyte cell line in the same period of time [29], or our effect was even higher than those described previously (~50%) [30]. These findings mean that adenosine is a potent stimulus for the release of VEGF when its extracellular bioavailability increases. Interestingly, it has been reported that angiotensin II levels were significantly higher in cell lysates and in the conditioned media of podocytes grown in high glucose conditions [31] when an increased VEGF expression was dependent on A_{2B} AR activation.

The involvement of A_{2B} receptor in VEGF production was also demonstrated in cell types like endothelial and smooth muscle cells exposed to hypoxia [32–34]. Transcrip-

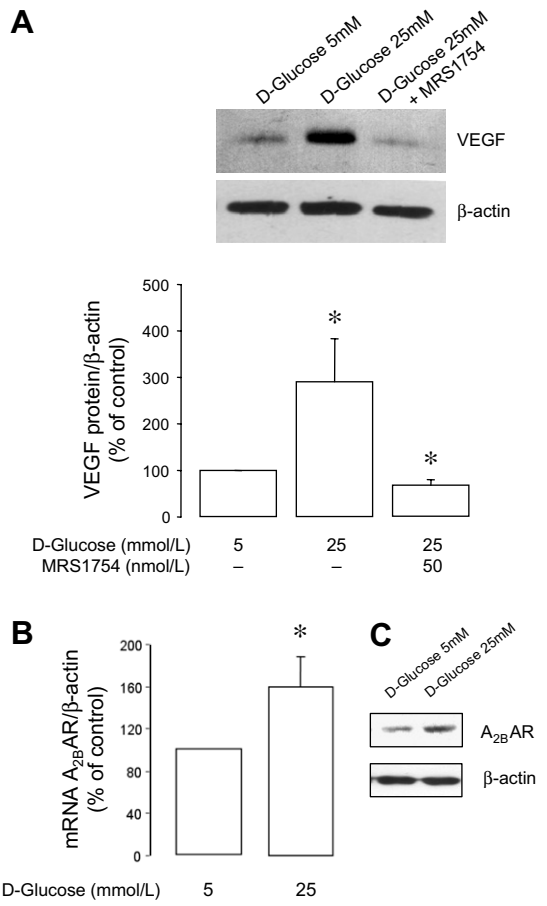

Fig. 4. Effects of high D-glucose concentration and A_{2B}AR activation on glomerular VEGF-A expression. (A) Western blot of dimeric VEGF and β actin in total protein extracts (100 μg) from exposed glomeruli to D-glucose concentration of 5 mM, 25 mM and 25 mM in the presence of 50 nM MRS1754 (selective A_{2B}AR antagonist) for 6 h. The graph shows the VEGF/β actin ratio immunosignals on Western blots. The VEGF/β actin ratio in glomeruli exposed to 5 mM D-glucose (control) was normalized to 100%. $^*P < 0.01$ versus control value. Mean ± SEM ($n = 5$). (B) Semiquantitative analysis of A_{2B}AR transcripts content in exposed glomeruli to 5 mM or 25 mM D-glucose. $^*P < 0.05$ versus control value. Mean ± SEM ($n = 3$). (C) Representative Western blot of A_{2B}AR (50 kDa) in exposed glomeruli to 5 mM or 25 mM D-glucose.

tional up-regulation in the promoter of the A_{2B}AR gene via hypoxia inducible factor-1alpha (HIF-1α) produces an increase in VEGF synthesis [35]. Interestingly, the use of cDNA microarrays demonstrated that HIF-1α was already up-regulated by hyperglycemia in parallel with an alteration of genes related to oxidative stress and glucose and lipid metabolism in mice glomeruli [36].

It seems that the role played by A_{2B} receptors could also be relevant in the maintenance of the constitutive VEGF levels in the glomerulus since the treatment with the MRS1754 antagonist significantly decreased VEGF production to levels even lower than control (Figs. 3 and 4). Similarly, when blocking the effect of high glucose concentrations with the antagonist, the VEGF content dropped again to levels lower than those considered basal. We observed that the agonist A_1 had a modest effect on lowering VEGF content in isolated glomeruli (Fig. 3A) which partially explains a decreased expression of this growth factor. The glomerulus is extremely sensitive to VEGF doses as shown by the series of glomerular injuries caused by the variation of the VEGF gene load [8,37] therefore, if the A_{2B} receptor can also modify the VEGF production then it would be an interesting matter to determine its role on the alterations of the glomerular function under normal and pathological conditions.

Acknowledgments

This study was supported by Grants 1070614 and 1070865 from FONDECYT (Chile), DI-UACH S-2006-67 from Universidad Austral de Chile (Chile) and C14060/50 from Andes Foundation (Chile).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.11.113.

References

[1] N. Ferrara, Vascular endothelial growth factor: basic science and clinical progress, Endocr. Rev. 25 (2004) 581–611.
[2] A. Tufro, V.F. Norwood, R.M. Carey, R.A. Gomez, Vascular endothelial growth factor induces nephrogenesis and vasculogenesis, J. Am. Soc. Nephrol. 10 (1999) 2125–2134.
[3] B.F. Schrijvers, A. Flyvbjerg, A.S. De Vriese, The role of vascular endothelial growth factor in renal pathophysiology, Kidney Int. 65 (2004) 2003–2017.
[4] V. Eremina, H.J. Baelde, S.E. Quaggin, Role of the VEGF-A signaling pathway in the glomerulus: evidence for crosstalk between components of the glomerular filtration barrier, Nephron Physiol. 106 (2007) 32–37.
[5] Y. Kitamoto, H. Tokunaga, K. Miyamoto, K. Tomita, VEGF is an essential molecule for glomerular structuring, Nephrol. Dial. Transplant. 17 (2002) 25–27.
[6] R. Foster, R. Hole, K. Anderson, S. Satchell, R. Coward, P. Mathieson, D. Gillatt, M. Saleem, D. Bates, S. Harper, Functional evidence that vascular endothelial growth factor may act as an autocrine factor on human podocytes, Am. J. Physiol. Renal Physiol. 284 (2003) 1263–1273.
[7] H. Sugimoto, Y. Hamano, D. Charytan, D. Cosgrove, M. Kieran, A. Sudhakar, R. Kalluri, Neutralization of circulating vascular endothelial growth factor by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria, J. Biol. Chem. 278 (2003) 12605–12608.
[8] V. Eremina, S. Cui, H. Gerber, N. Ferrara, J. Haigh, A. Nagy, M. Ema, J. Rossant, S. Jothy, J.H. Miner, S. Quaggin, Vascular endothelial growth factor a signaling in the podocyte-endothelial compartment is required for mesangial cell migration and survival, J. Am. Soc. Nephrol. 17 (2006) 724–735.
[9] E. Liu, M. Morimoto, S. Kitajima, T. Koike, Y. Yu, H. Shiiki, M. Nagata, T. Watanabe, J. Fan, Increased expression of vascular endothelial growth factor in kidney leads to progressive impairment of glomerular functions, J. Am. Soc. Nephrol. 18 (2007) 2094–2104.
[10] D. Shweiki, A. Itin, D. Soffer, E. Keshet, Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis, Nature (London) 359 (1992) 843–845.

[11] S. Wakelin, L. Marson, S. Howie, J. Garden, J. Lamb, J. Forsythe, The role of vascular endothelial growth factor in the kidney in health and disease, Nephron Physiol. 98 (2004) 73–79.
[12] S. Hoshi, Y. Shu, F. Yoshida, T. Inagaki, J. Sonoda, T. Watanabe, K. Nomoto, M. Nagata, Podocyte injury promotes progressive nephropathy in Zucker diabetic fatty rats, Lab. Invest. 82 (2002) 25–35.
[13] M. Iglesias-De la Cruz, F. Ziyadeh, M. Isono, M. Kouahou, D. Han, R. Kalluri, P. Mundel, S. Chen, Effects of high glucose and TGF-beta1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes, Kidney Int. 62 (2002) 901–913.
[14] V. Vallon, B. Mühlbauer, H. Osswald, Adenosine and kidney function, Physiol. Rev. 86 (2006) 901–940.
[15] J. Foidart, C. Dechenne, P. Mahieu, C. Creutz, J. De Mey, Tissue culture of normal rat glomeruli. Isolation and morphological characterization of two homogeneous cell lines, Invest. Cell Pathol. 2 (1979) 15–26.
[16] B. Fredholm, A. Ijzerman, K. Jacobson, K. Klotz, J. Linden, International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors, Pharmacol. Rev. 53 (2001) 527–552.
[17] P. Casanello, A. Torres, F. Sanhueza, M. Gonzalez, M. Farias, V. Gallardo, M. Pastor-Anglada, R. San Martin, L. Sobrevia, Equilibrative nucleoside transporter 1 expression is downregulated by hypoxia in human umbilical vein endothelium, Circ. Res. 97 (2005) 16–24.
[18] R. San Martín, P. Cáceres, R. Azócar, M. Alvarez, A. Molina, M. Vera, M. Krauskopf, Seasonal environmental changes modulate the prolactin receptor expression in an eurythermal fish, J. Cell. Biochem. 92 (2004) 42–52.
[19] G. Vasquez, F. Sanhueza, R. Vasquez, M. Gonzalez, R. San Martin, P. Casanello, L. Sobrevia, Role of adenosine transport in gestational diabetes-induced L-arginine transport and nitric oxide synthesis in human umbilical vein endothelium, J. Physiol. 560 (2004) 111–122.
[20] S. Yamaguchi, S. Umemura, K. Tamura, T. Iwamoto, N. Nyui, T. Ishigami, M. Ishii, Adenosine A_1 receptor mRNA in microdissected rat nephron segments, Hypertension 26 (1995) 1181–1185.
[21] H. Vitzthum, B. Weiss, W. Bachleitner, B.K. Krämer, A. Kurtz, Gene expression of adenosine receptors along the nephron, Kidney Int. 65 (2004) 1180–1190.
[22] T. Pawelczyk, M. Grden, R. Rzepko, M. Sakowicz, A. Szutowicz, Region-specific alterations of adenosine receptors expression level in kidney of diabetic rat, Am. J. Pathol. 167 (2005) 315–325.
[23] D. Cha, N. Kim, J. Yoon, S. Jo, W. Cho, H. Kim, N. Won, Role of vascular endothelial growth factor in diabetic nephropathy, Kidney Int. 77 (2000) 104–112.
[24] D. Cha, Y. Kang, S. Han, Y. Jee, K. Han, J. Han, Y. Kim, N. Kim, Vascular endothelial growth factor is increased during early stage of diabetic nephropathy in type II diabetic rats, J. Endocrinol. 183 (2004) 183–194.
[25] G. Munoz, R. San Martin, M. Farias, L. Cea, A. Vecchiola, P. Casanello, L. Sobrevia, Insulin restores glucose inhibition of adenosine transport by increasing the expression and activity of the equilibrative nucleoside transporter 2 in human umbilical vein endothelium, J. Cell. Physiol. 209 (2006) 826–835.
[26] M. Sakowicz, A. Szutowicz, T. Pawelczyk, Differential effect of insulin and elevated glucose level on adenosine transport in rat B lymphocytes, Int. Immunol. 17 (2005) 145–154.
[27] D.Y. Huang, V. Vallon, H. Zimmermann, P. Koszalka, J. Schrader, H. Osswald, Ecto-5′-nucleotidase (cd73)-dependent and -independent generation of adenosine participates in the mediation of tubuloglomerular feedback in vivo, Am. J. Physiol. Renal Physiol. 291 (2006) F282–F288.
[28] A. Nishiyama, A. Miyatake, Y. Aki, T. Fukui, M. Rahman, S. Kimura, Y. Abe, Adenosine A_1 receptor antagonist KW-3902 prevents hypoxia-induced renal vasoconstriction, J. Pharmacol. Exp. Ther. 291 (1999) 988–993.
[29] Y. Kang, Y. Park, B. Kim, S. Han, Y. Jee, K. Han, M. Lee, H. Song, D. Cha, S. Kang, D. Han, Angiotensin II stimulates the synthesis of vascular endothelial growth factor through the p38 mitogen activated protein kinase pathway in cultured mouse podocytes, J. Mol. Endocrinol. 36 (2006) 377–388.
[30] S. Chen, J. Lee, M. Iglesias-De la Cruz, A. Wang, A. Izquierdo-Lahuerta, N. Gandhi, F. Danesh, G. Wolf, F. Ziyadeh, Angiotensin II stimulates alpha3(IV) collagen production in mouse podocytes via TGF-beta and VEGF signalling: implications for diabetic glomerulopathy, Nephrol. Dial. Transplant. 20 (2005) 1320–1328.
[31] T. Yoo, J. Li, J. Kim, D. Jung, S. Kwak, D. Ryu, H. Choi, J. Kim, H. Kim, S. Han, J. Lee, D. Han, S. Kang, Activation of the renin-angiotensin system within podocytes in diabetes, Kidney Int. 71 (2007) 1019–1027.
[32] M.B. Grant, R.W. Tarnuzzer, S. Caballero, M.J. Ozeck, M.I. Davis, P.E. Spoerri, I. Feoktistov, I. Biaggioni, J.C. Shryock, L. Belardinelli, Adenosine receptor activation induces vascular endothelial growth factor in human retinal endothelial cells, Circ. Res. 85 (1999) 699–706.
[33] I. Feoktistov, A.E. Goldstein, S. Ryzhov, D. Zeng, L. Belardinelli, T. Voyno-Yasenetskaya, I. Biaggioni, Differential expression of adenosine receptors in human endothelial cells: role of A_{2B} receptors in angiogenic factor regulation, Circ. Res. 90 (2002) 531–538.
[34] I. Feoktistov, S. Ryzhov, H. Zhong, A.E. Goldstein, A. Matafonov, D. Zeng, I. Biaggioni, Hypoxia modulates adenosine receptors in human endothelial and smooth muscle cells toward an A_{2B} angiogenic phenotype, Hypertension 44 (2004) 649–654.
[35] T. Kong, K. Westerman, M. Faigle, H. Eltzschig, S. Colgan, HIF-dependent induction of adenosine A_{2B} receptor in hypoxia, FASEB J. 20 (2006) 2242–2250.
[36] H. Makino, Y. Miyamoto, K. Sawai, K. Mori, M. Mukoyama, K. Nakao, Y. Yoshimasa, S. Suga, Altered gene expression related to glomerulogenesis and podocyte structure in early diabetic nephropathy of db/db mice and its restoration by pioglitazone, Diabetes 55 (2006) 2747–2756.
[37] V. Eremina, M. Sood, J. Haigh, A. Nagy, G. Lajoie, N. Ferrara, H.P. Gerber, Y. Kikkawa, J.H. Miner, S.E. Quaggin, Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases, J. Clin. Invest. 111 (2003) 707–716.