



## Altered expression of Aquaporin-2 in one-kidney, one-clip hypertension

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### ABSTRACT

**Aims:** The aim of the present study was to evaluate the regulation of Aquaporin-2 (AQP2) water channel in the kidney of one-kidney, one-clip rats (Goldblatt-1 model). In addition, some mechanisms that underlie the role of AQP2 in the Goldblatt-1 model were evaluated.

**Main methods:** Sprague-Dawley rats were divided in three groups: control two-kidney, no clip (C, 2K-NC); nephrectomized one-kidney, no clip (N, 1K-NC) and Goldblatt one-kidney, one-clip (G, 1K-1C). AQP2 expression (by westernblot, real time PCR, immunohistochemistry and immunofluorescence), vasopressin V2 receptor expression (by real time PCR), cAMP concentration, NFkB and TonEBP (cytosol to nucleus ratio) were evaluated in the renal medulla.

**Key findings:** AQP2 expression, V2 receptor expression and cAMP concentration were decreased in the renal medulla of 1K-1C rats, NFkB translocation was favoured towards the nucleus suggesting its activation while TonEBP translocation was not altered in this model of hypertension.

**Significance:** In this model of hypertension the decrease of AQP2 expression could be a mechanism that counteracts the high blood pressure promoting water excretion and this may be consequence of decreased vasopressin sensitivity and/or the increased activity of NFkB at renomedullary collecting duct level. Given that renovascular hypertension is among the most common causes of secondary hypertension, it is important to elucidate all the relevant mechanisms involved in the generation or in the compensation of the hypertensive state in order to improve the diagnoses and treatment of the patients.

### 1. Introduction

The kidney plays a central role in the regulation of salt and water balance, contributing to the control of arterial blood pressure. This regulation is achieved through the activity of both sodium and water transporters. This work has a special focus on water transport mediated by the aquaporins and in particular aquaporin-2 (AQP2). AQP2 plays a central role in the final regulation of water reabsorption in the renal collecting duct, being vasopressin (AVP) the main regulator of this process [1]. AVP exerts its effects acting through three different receptors: V1a, V1b (or V3) and V2 [2]. V1b receptors are present at central nervous system level, being involved in cognitive functions in the brain. Both V1a and V2 receptors are present in the kidney. V1a is

associated with the vasa recta and is probably present in the intercalated cells of the cortical collecting duct. V2 receptor, the unique AVP receptor that signals through cAMP, has the key function of controlling fluid homeostasis [3]. Binding of AVP to V2 receptors in the basolateral membrane of principal cells in the collecting ducts stimulates adenylate cyclase producing cAMP, whose effects are mainly mediated by protein kinase A (PKA) [4, 5].

AVP regulates water permeability in the renal collecting duct by both short and long-term mechanisms. Short-term regulation occurs as a result of the modulation of trafficking of AQP2-containing membrane vesicles to and from the apical plasma membrane and long-term regulation occurs as a result of AQP2 expression regulation [6, 7].

Other proteins have been described as regulators of AQP2

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expression, such as transcription factor tonicity responsive enhancer binding protein (TonEBP) [8] and the inflammatory mediator nuclear factor-kappaB (NFkB) [9]. TonEBP is a transcription factor activated under hypertonic conditions to protect renal cells from hypertonic stress stimulating the transcription of specific proteins [10]. Inflammation is a key feature in the initiation, progression and clinical implication of several cardiovascular diseases and the inflammatory response is essential in the pathogenesis of hypertension [11–13].

In this pathology, focus has been made on sodium transport but not on water transport, despite the importance of renal water handling in this pathological condition. There are no conclusive studies about the state of AQP2 during hypertension. However, some studies demonstrated changes in AQP2 targeting and expression in different experimental models of hypertension.

An increased expression and apical targeting of AQP2 in association with enhanced activity of the AVP/cAMP pathway has been reported in spontaneously hypertensive rats (SHR) [14–16]. On the other hand, a decrease in AQP2 expression was described in a Goldblatt-2 model of hypertension (two-kidney, one-clip; 2 K-1C) [17, 18]. However, there are no reports on the status of AQP2 in the Goldblatt-1 model of hypertension (one-kidney, one-clip; 1 K-1C). This renovascular model of hypertension described by Goldblatt is appropriate for studying the role of volume expansion in the development of hypertension [19, 20]. Since in the model of 1 K-1C there is no other kidney, there is no pressure diuresis and natriuresis, there is rapid salt and water retention and hypertension soon becomes volume dependent and plasma renin activity usually remains normal after a short increase following the surgery [21, 22].

On these bases, the aim of the present study was to evaluate the regulation of AQP2 water channels in the kidney of one-kidney-one-clip rats. In addition, some mechanisms that underlie the role of AQP2 in the Goldblatt-1 model were evaluated.

## 2. Materials and methods

### 2.1. Animals and hypertension model

Male Sprague Dawley rats were purchased from Facultad de Ciencias Veterinarias -Universidad de Buenos Aires. Protocols were in compliance with ARRIVE guidelines, were designed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Committee for Use and Care of Laboratory Animals (CICUAL) from Facultad de Farmacia y Bioquímica. Forty-five-day-old rats weighing 150 g were anesthetized with Ketamine/Xylazine (50–10, mg/Kg of body weight), the left kidney was exposed and a 0.25 mm gap silver clip was placed around the renal artery. For right nephrectomy, two ligatures were made around the renal vascular pedicle and the ureter. The kidney was then removed without the adrenal gland (Goldblatt rats). In another group of rats only the right kidney was extracted (nephrectomized rats) and a third group was subjected to a sham surgery procedure, with the exception of the artery clip and the nephrectomy (Control rats). All rats received 0.1 ml (40,000 IU) of amoxicillin at the end of the surgery.

Therefore we worked with three groups: control two-kidney-no clip (C, 2 K-NC,  $n = 12$ ); nephrectomized one-kidney-no clip (N, 1 K-NC  $n = 12$ ) and Goldblatt hypertensive group one-kidney-one-clip (G, 1 K-1C,  $n = 15$ ), which were studied four weeks after surgery.

### 2.2. Determination of systolic blood pressure

Systolic BP was recorded weekly in conscious rats by tail plethysmography (ADInstruments PowerLab 8/30 and NIBP Controller ML125).

### 2.3. Determinations in the 24-h metabolic cage studies

Animals were acclimatized to metabolic cages for 2 days before 24 h urine collection. Urine samples were analyzed for total protein concentration (Proti U/LCR Kit; Wiener Lab., Rosario, Argentina) and urine osmolality (OSMETTE, Precision Systems Inc., MA, USA). Urine volume was measured gravimetrically. Kinetic determinations of serum and urinary creatinine concentration were evaluated using a kit provided by Wiener. Urinary sodium was measured using an ion analyzer (TecnoLab; Mod. T-412, Argentina).

Blood collection was performed with the rats under urethane anesthesia (1 g/Kg via i.p) by cardiac puncture; animals were killed by exsanguination, then both kidneys or the remaining kidney were immediately removed and weighed.

### 2.4. Tissue processing for Western blot analysis in medulla homogenates

Immediately after the animals were sacrificed, their kidneys were isolated and the renal medulla was dissected and homogenized in an appropriate homogenization buffer (250 mmol/l sucrose, 1 mmol/l EDTA, 0.1 mmol/l PMSF and 10 mmol/l Tris-ClH, pH 7.6. Large tissue debris and nuclear fragments were removed by a low-speed spin (1000 g, 10 min, 4 °C). Protein concentration was measured using BCA TM Protein Assay Kit (Pierce, Rockford, IL, USA).

### 2.5. Western blot for AQP2

Immunoblotting analysis was used to identify AQP2. AQP2 antibody (rabbit anti-rat AQP2; Santa Cruz Biotechnology, Inc., CA, USA) was followed by a donkey anti-rabbit IgG (HRP) secondary antibody (Abcam Inc., Cambridge, MA, USA) and revealed using Bio-Lumina kit (Kalium Technologies; Buenos Aires, Argentina). Beta-tubulin was used as loading control (Abcam). AQP2 antibody recognizes at least two bands of 29 and 35–40 kDa corresponding to unglycosylated and glycosylated AQP2, respectively.

The relative protein levels were analyzed with Gel Pro Analyzer 3.1 for Windows and the ratio of AQP2 to beta-tubulin was calculated.

### 2.6. Tissue processing for Western blot analysis in cytosolic fraction and purified nuclei

After homogenization of the renal medulla, the homogenate was centrifuged for 10 min at  $800 \times g$  to obtain the nuclear fraction in the pellet and the cytosolic fraction in the supernatant. The pellet was washed with homogenization buffer and suspended in a solution of 2.4 M sucrose followed by a centrifugation at  $50000 \times g$  for 1 h to obtain the purified nuclei in the pellet.

### 2.7. Western blots for NFkB and TonEBP

Immunoblotting analysis was used to identify NFkB or TonEBP in the cytosolic fraction and in purified nuclei. The appropriate primary antibody (mouse anti-rat NFkB, Santa Cruz Biotechnology, or rabbit anti-rat TonEBP; Santa Cruz Biotechnology) was followed by a secondary antibody (Horse anti mouse HRP, GE Healthcare, UK or donkey anti-rabbit IgG HRP Abcam) and revealed as described above. NFkB antibody recognizes a 65-kDa band corresponding to p65 subunit and TonEBP antibody recognizes a 170-KDa band.

Beta-tubulin was used as loading control for the cytosolic fraction and Lamina (Mouse anti rat Millipore MA, USA) was used as loading control for the purified nuclei. The relative protein levels were analyzed with Gel Pro Analyzer 3.1 for Windows and the ratio of cytosolic to nuclei expression was used as an indicator of transcription factor activation.

## 2.8. Real-time PCR for AQP2 and V2 receptor

Total RNA was isolated using the SV total RNA Isolation System (Promega) and reverse transcribed to cDNA using a high capacity reverse transcription kit (A&B, MA, USA). For real-time detection of AQP2 and V2 transcripts and the reference gene (GAPDH), MezclaReal (Biodynamics, Buenos Aires, Argentina) and specific primers were used [23, 24]. The normalized gene expression method ( $2^{-\Delta\Delta CT}$ ) for the relative quantification of gene expression was used [25].

## 2.9. cAMP measurements

cAMP was measured in the renal medulla. Approximately 100 mg of renal medulla was homogenized in ice-cold absolute ethanol and centrifuged for 15 min at 1200 g. The supernatant was dried, and the remaining residue was suspended for cAMP determination by competition of [3H]-cAMP for PKA [26]. Results were expressed as pmoles/mg of protein.

## 2.10. Immunohistochemistry for AQP2

Five micrometer slices were incubated with primary rabbit polyclonal antibody to AQP2 (Abcam) and a secondary biotinylated donkey anti rabbit (Jackson ImmunoResearch, West Grove, PA, USA), followed by the streptavidin-biotin-peroxidase reaction (Dako Cytomation, Glostrup, Denmark) visualized by exposure to diaminobenzidine (DAB)-H<sub>2</sub>O<sub>2</sub>. Endogenous peroxidase activity was quenched with hydrogen peroxide to prevent unspecific staining. Tissue sections were counterstained with hematoxylin.

## 2.11. Immunofluorescence for AQP2

Kidneys were fixed with 4% paraformaldehyde, pH 7.4, cryoprotected in 5% and 25% sucrose/PBS, pH 7.4 and frozen at  $-70^{\circ}\text{C}$ . Then the kidneys were cut in 5  $\mu\text{m}$  slices. Kidney sections were incubated with a primary rabbit polyclonal antibody to AQP2 (Abcam) followed by a secondary FITC labeled donkey anti rabbit antibody (Alexa fluor 488, goat anti rabbit Ig G; Invitrogen) and mounted with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA, USA).

## 2.12. Image capture and analysis

All determinations were performed blindly and under similar light, gain and offset conditions by the same operator.

Images from immunohistochemical sections were captured using a Nikon Alphaphot-2 YS2 light microscope (Nikon Instrument Group, Melville, NY), coupled to a Sony color video camera digital (Model No. SSC-DC50A).

Images from immunofluorescence were examined by wide-field microscopy with an epifluorescence Nikon Eclipse Ti microscope. Images were taken with the acquisition freeware Micrometric SE premium.

## 2.13. Statistics

One-way ANOVA with Bonferroni's post-test for multiple comparisons was performed using Graph Pad Prism version 5.0 for Windows.

## 3. Results

### 3.1. Characterization of the animal model

Data for renal functional parameters and systolic BP 4 weeks after the surgical procedure are shown in Table 1.

Systolic BP was significantly higher in 1 K-1C group compared to control and 1 K-NC groups ( $p < 0.001$ ). Kidney weight/100 g b.w.

increased in both 1 K-NC ( $p < 0.01$ ) and 1 K-1C ( $p < 0.001$ ) groups compared to the control group meanwhile there were no differences in body weight between the three groups. Kidney size increases in a number of physiological and pathological situations, including uninephrectomy. Renal mass increases after nephrectomy mainly in the cortex, particularly in the proximal convoluted tubules, and appears to be of a hypertrophic, rather than hyperplastic nature and is mediated by a cell cycle-dependent mechanism [27].

Urinary volume significantly increased in 1 K-1C group compared to control group ( $p < 0.05$ ), meanwhile only had a tendency to increase in 1 K-NC group. Urinary osmolality decreased in 1 K-1C group compared to both 1 K-NC and the control group ( $p < 0.05$ ).

Proteinuria significantly increased in 1 K-1C group when compared to control group ( $p < 0.05$ ). There were no significant differences in creatinine clearance and urinary sodium excretion among the three groups.

### 3.2. Aquaporin-2 expression is decreased in the renal medulla of the hypertensive 1 K-1C rats

Fig. 1 shows that both AQP2 protein and mRNA expression were decreased in the renal medulla of 1 K-1C rats compared to both 1 K-NC and control groups ( $p < 0.05$ ). We evaluated AQP2 expression both at protein and mRNA levels because uncoupled expression between AQP2 mRNA and protein has been described under certain conditions [28].

The results of AQP2 Western blot were corroborated by immunohistochemistry and immunofluorescence (Fig. 2). The images obtained by immunofluorescence show AQP2 labeling both in the cytosol and in the apical membranes of the principal cells for the three groups, there were no net changes in AQP2 translocation in none of the studied groups.

### 3.3. V2 receptor expression and cAMP production are decreased in the renal medulla of hypertensive 1 K-1C rats

V2 receptor mRNA expression decreased in the renal medulla of 1 K-1C rats compared to both 1 K-NC and control groups ( $p < 0.01$ ), as can be shown in Fig. 3A.

Fig. 3B shows that cAMP production decreased in the renal medulla of 1 K-1C rats compared to both 1 K-NC and control groups ( $p < 0.01$ ).

### 3.4. NFkB is activated in hypertensive 1 K-1C rats

Fig. 4A shows that cytosolic/nuclear ratio of NFkB expression decreased in hypertensive 1 K-1C rats, due to the increase in NFkB nuclear localization. NFkB was shown to negatively regulate AQP2 expression, so this activation of NFkB could be contributing to AQP2 down-regulation.

### 3.5. TonEBP translocation is not altered in the renal medulla of hypertensive 1 K-1C rats

Fig. 4B shows that cytosolic/nuclear ratio of TonEBP expression did not change in 1 K-1C animals when compared to control group or to the nephrectomized group, indicating that TonEBP is not activated in this model of hypertension.

## 4. Discussion

In this work we show that AQP2 expression is altered in 1 K-1C hypertension with a decrease in both protein expression and mRNA levels. The decrease in AQP2 expression results in an altered urinary concentration that leads to an increased urinary volume and a decreased urinary osmolality in 1 K-1C hypertensive group. The fact that AVP pathway is also altered may explain, at least in part, the diminished AQP2 expression and the increased diuresis found in this

**Table 1**  
Systolic blood pressure and renal functional parameters.

	2 K-NC	1 K-NC	1 K-1C
Systolic arterial pressure (mm Hg)	124.7 ± 0.9 (n = 7)	123.6 ± 1.0 (n = 7)	185.0 ± 15.9 <sup>***##</sup> (n = 7)
Kidney weight (g)	1.46 ± 0.04 (n = 12)	2.13 ± 0.10 <sup>***</sup> (n = 12)	2.26 ± 0.13 <sup>***</sup> (n = 15)
Body weight (g)	348.1 ± 15.8 (n = 11)	331.0 ± 22.0 (n = 11)	320.6 ± 12.6 (n = 14)
Kidney weight/100 g b.w. (g/100 g)	0.35 ± 0.03 (n = 11)	0.59 ± 0.02 <sup>**</sup> (n = 11)	0.67 ± 0.05 <sup>***</sup> (n = 14)
Urinary volume (ml/24 h)	8.8 ± 1.1 (n = 6)	13.3 ± 1.8 (n = 10)	18.1 ± 2.5 <sup>*</sup> (n = 10)
Urinary Osmolality (mOsm/L)	1870 ± 210 (n = 6)	1910 ± 169 (n = 6)	964 ± 245 <sup>##</sup> (n = 7)
Urinary sodium (meq/24 h)	2.98 ± 0.95 (n = 6)	3.44 ± 0.88 (n = 6)	2.88 ± 0.47 (n = 7)
Proteinuria (mg/24 h/100 g)	3.12 ± 0.60 (n = 6)	4.41 ± 0.42 (n = 6)	5.67 ± 0.72 <sup>*</sup> (n = 6)
Creatinine clearance (ml/min/100 g)	0.37 ± 0.07 (n = 6)	0.32 ± 0.02 (n = 6)	0.37 ± 0.10 (n = 6)

2 K-NC: control group; 1 K-NC: one kidney-no clip; 1 K-1C: one kidney-1 clip hypertensive group. One-way ANOVA followed by Bonferroni's test for multiple comparisons.

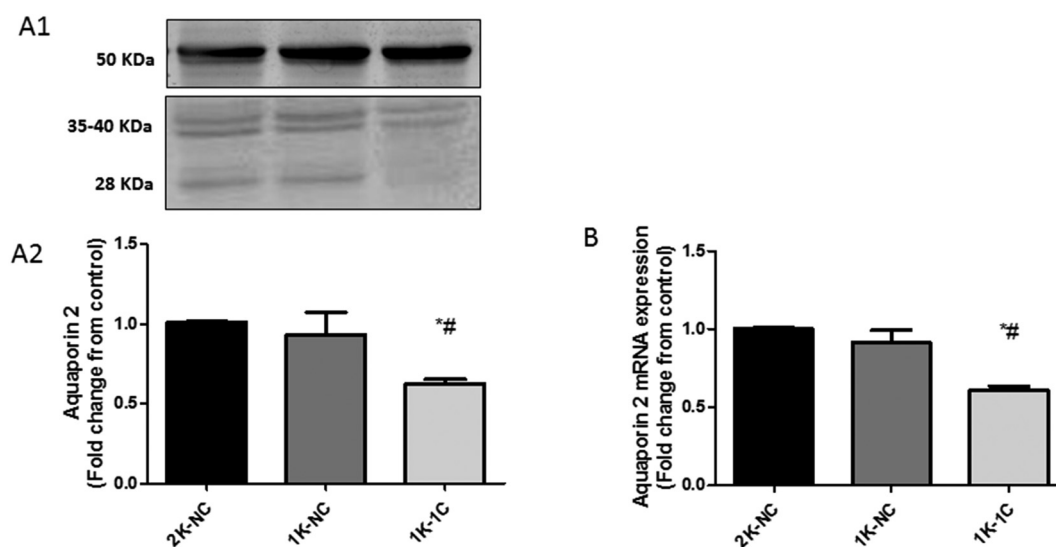
\* p < 0.05 vs 2 K-NC.

\*\* p < 0.01 vs 2 K-NC.

\*\*\* p < 0.001 vs. 2 K-NC.

# p < 0.05 vs. 1 K-NC.

### p < 0.001 vs. 1 K-NC. Data are mean ± SEM.



**Fig. 1.** Aquaporin-2 expression in homogenates of renal medulla

A1. Representative Western Blot analysis of AQP2 (28 and 35–40 kDa) and tubulin (50 kDa) in homogenates of renal medulla. A2. AQP2 protein expression indicated as AQP2/tubulin ratio fold change from 2 k-NC control rats. B. AQP2 mRNA levels are expressed as relative values from 2 K-NC control rats. The following formula was applied:  $\Delta\Delta CT = (CTAQP2-CTGAPDH)_{\text{experimental}} - (CTAQP2-CTGAPDH)_{\text{2 K-NC control rats}}$ .

2 K-NC: control group; 1 K-NC: one kidney, no clip; 1 K-1C: one kidney, 1 clip hypertensive group. One-way ANOVA followed by Bonferroni's test for multiple comparisons. \*p < 0.05 vs 2 K-NC, #p < 0.05 vs 1 K-NC rats. Data are mean ± SEM (n = 6).

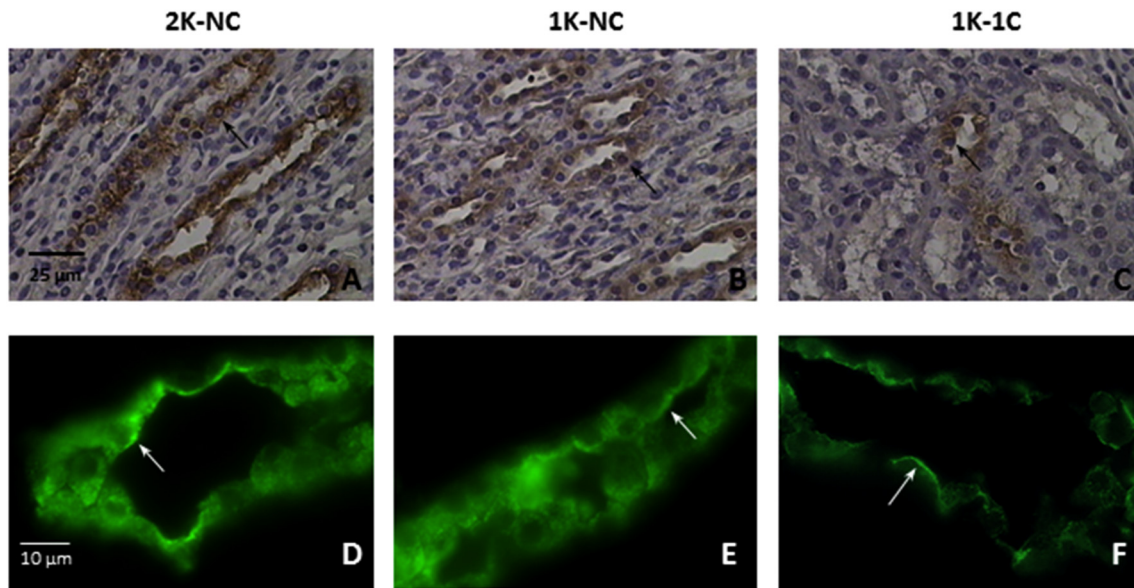
hypertension model. These results are in accordance with those reported by Lee et al., who also showed a reduced expression of AQP2 without changes in its translocation and a diminished cAMP production in the clipped kidney from 2 K-1C rats, another model of Goldblatt renovascular hypertension [17].

Contrary to results mentioned above, it was reported that the medullary expression and apical targeting of AQP2 and adenylate cyclase activity are increased in the kidney of SHR, contributing to the pathogenesis of hypertension [14, 16]. Similarly, in the kidney of DOCA-salt hypertensive rats, a model where hypertension maintenance has been attributed to increased AVP, cAMP and AQP2 expression and shuttling are increased suggesting that they are causally related with the development of hypertension [29].

In this work we show a decrease in V2 receptor expression and cAMP reduction in the renal medulla of 1 K-1C rats. AVP plays an important role in the regulation of BP by means of V1 and V2 receptors; however the regulation of the expression of these receptors in hypertension is not completely understood. Burrell et al. demonstrated that in SHR, increase of plasma AVP and activation of renal V1a

receptor occurs during developing hypertension, with downregulation when hypertension is established [24]. Contrary to our results, they did not observe changes in V2 receptor expression, but the study was conducted in a different model of hypertension [17, 30]. Some authors have shown that plasma AVP is elevated in rats with 1 K-1C Goldblatt hypertension [31, 32]. Share et al. pointed out that AVP may contribute to 1 K-1C hypertension by virtue of its antidiuretic action, allowing the expansion of blood volume that apparently is necessary for the chronic stage of the hypertensive state [33]. Lariviere et al. showed that together with an increased concentration of AVP in plasma, the number of vascular AVP-binding sites is decreased in 1 K-1C hypertensive rats [32]. So it is possible that increased AVP levels leads to a decrease in renal medullary V2 receptor expression as a compensatory mechanism, diminishing AVP sensitivity and consequently decreasing cAMP levels and AQP2 expression in order to counteract the volume expansion.

On the other hand, the reduction in cAMP production and consequently AQP2 decrease may be consequence of the ischemic condition of the clipped kidney [17, 34], which in turn promotes oxidative stress. It is known that an increase in oxidative stress plays a role in



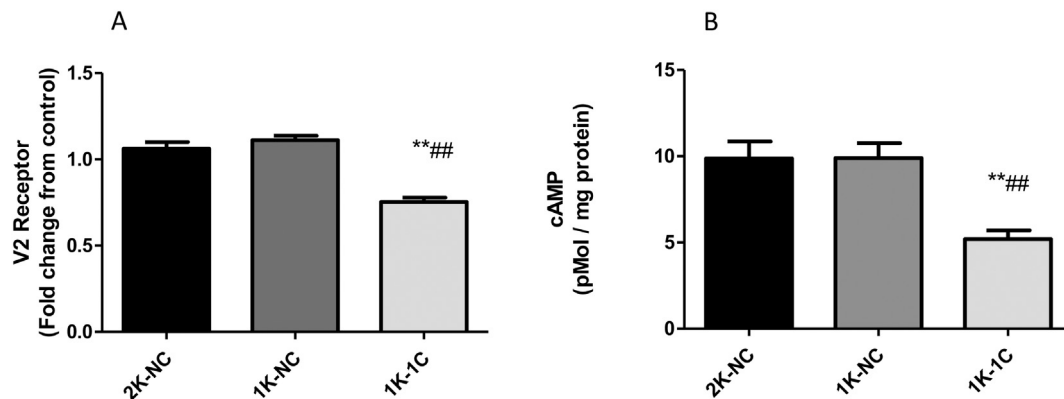
**Fig. 2.** Aquaporin-2 protein expression in histological sections  
 A–C: Representative images of immunohistochemical staining for AQP2. All images are in the same magnification: 400×. Each arrow shows a representative collecting duct principal cell. D–F: Representative images of Immunofluorescence staining for AQP2. All images are in the same magnification: 600×. Each arrow shows apical staining for AQP2.  
 2 K-NC: control group; 1 K-NC: one kidney, no clip; 1 K-1C: one kidney, 1 clip hypertensive group; (n = 3).

renovascular hypertension [35, 36] and that the interaction between reactive oxygen species (ROS) and NO reduces its bioavailability [37]. In a previous work we demonstrated that AQP2 is down-regulated in the renal medulla of rats made hypertensive by chronic inhibition of nitric oxide synthase [38] and later we provided evidence that NO has a role in the control of AQP2 expression [39]. Besides the decrease in NO bioavailability, the interaction of ROS with NO promotes the increase in pro-oxidant peroxynitrite. The combination of diminished NO bioavailability and increased peroxynitrite impairs intrarenal vascular, glomerular and tubular function [37].

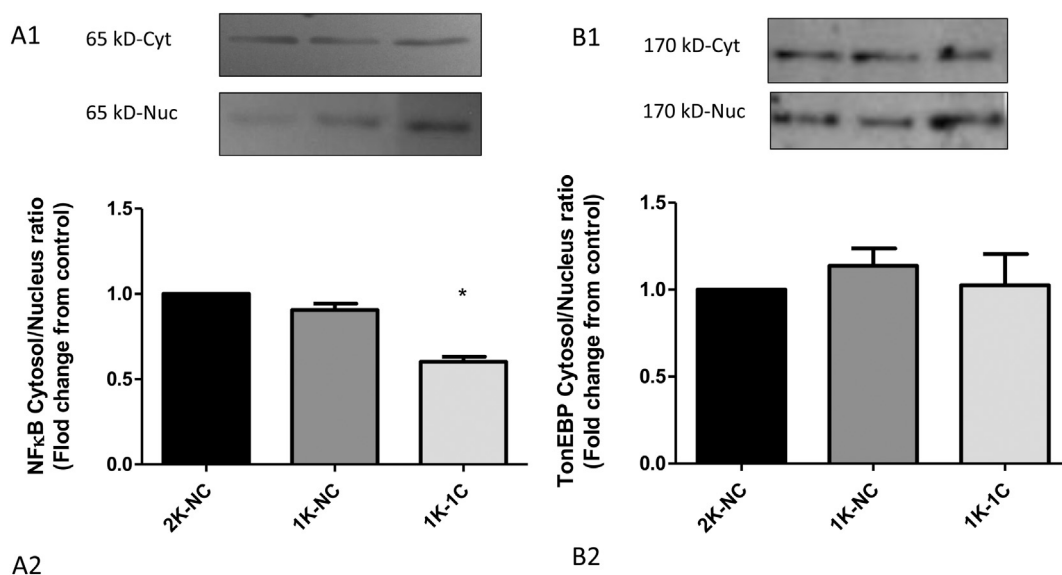
TonEBP, a protein known to regulate AQP2 expression by enhancing its gene transcription independently of AVP action, is not activated in this model of hypertension, since this is a volume-dependent hypertension model and TonEBP is activated in hypertonic environments [40].

As expected, NFκB is activated in this model of hypertension which courses with the development of pro-inflammatory mechanisms. It was already shown that inflammatory processes contribute to the increased

arterial pressure in human essential arterial hypertension and in different animal models of experimental hypertension [41]. Besides, it is known that NFκB activation is important for the downregulation of AQP2 channel and vasopressin V2 receptor expression during sepsis [42] and other inflammatory diseases [9]. Hasler et al. identified NFκB as a negative acting transcription factor that physiologically controls AQP2 transcription and may antagonize both CREB and TonEBP stimulatory effects. In that article the authors said that the strong activation of NFκB observed in renal inflammatory diseases is most likely to account for the observed decrease of AQP2 expression and accompanies urinary concentration defects [9]. The analysis of AQP2 promoter revealed two putative κB binding elements. NFκB stimulates or represses target gene transcription depending on the nature of the bound NFκB homo- or heterodimer and on bound NFκB cofactors [9]. The differential dimerization between NFκB family members allows for cell-specific transcriptional modulation of target genes in response to a great number of stimuli [9]. Hasler et al. showed that LPS treatment of cultured mouse CD principal cells or rat kidney slices decreased AQP2



**Fig. 3.** V2 receptor expression and cAMP concentration in renal medulla  
 A: V2 receptor mRNA levels are expressed as relative values from 2 K-NC control rats. The following formula was applied:  $\Delta\Delta CT = (CTV2R - CTGAPDH)_{\text{experimental}} - (CTV2R - CTGAPDH)_{\text{2 K-NC control rats}}$ . B: cAMP concentration assessed by competition of [<sup>3</sup>H]-cAMP for PKA.  
 2 K-NC: control group; 1 K-NC: one kidney, no clip; 1 K-1C: one kidney, 1 clip hypertensive group. One-way ANOVA followed by Bonferroni's test for multiple comparisons. \*\*p < 0.01 vs. 2 K-NC, ## p < 0.01 vs. 1 K-NC rats. Data are mean ± SEM (n = 4).



**Fig. 4.** NFKB and TonEBP cytosolic/nuclear ratio in renal medulla

A1: Representative western blot analysis of NFKB p65 (65 kDa) in cytosolic fraction and purified nuclei of renal medulla. A2: NFKB activation indicated as cytosolic/nuclear ratio fold change from 2K-NC control rats. B1: Representative western blot analysis of TonEBP (170 kDa) in cytosolic fraction and purified nuclei of renal medulla. B2: TonEBP cytosolic/nuclear ratio fold change from 2K-NC control rats.

2K-NC: control group; 1K-NC: one kidney, no clip; 1K-1C: one kidney, 1 clip hypertensive group. One-way ANOVA followed by Bonferroni's test for multiple comparisons. \* $p < 0.05$  vs. 2K-NC. Data are mean  $\pm$  SEM ( $n = 3$ ).

expression, being this effect mediated by decreased p65 and increased p50 and p52 binding to kB elements of AQP2 promoter [9]. This result is opposite to our finding because we found that in 1K-1C hypertensive Goldblatt model AQP2 expression decreases as a consequence of increased nuclear localization of p65. However, in accordance to us Lin et al. found that knockdown of NF-kBp65 or NF-kBp65 inhibition by pyrrolidine dithiocarbamate (PDTC) enhanced AQP2 expression in IMCD cells exposed to TNF- $\alpha$  [43].

## 5. Conclusions

Our results allow us to conclude that, in Goldblatt-1 hypertensive model, the decrease of AQP2 could be counterbalancing the enhancement in BP by increasing water excretion as a consequence of the decrease in vasopressin sensitivity, although this needs further investigation. On the other hand, we cannot discard activation of NFKB as a mechanism contributing to AQP2 downregulation although it would be hard to conceive inflammation as a compensatory mechanism for hypertension since it is actually one of the initiating factors.

Hypertension is one of the leading causes of morbidity and mortality and increases every year. Renovascular hypertension is among the most common causes of secondary hypertension [37]. Therefore, it is very important to elucidate all the relevant mechanisms involved in the generation or in the compensation of the hypertensive state in order to improve the diagnoses and treatment of the patients.

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## Conflict of interest

The authors declare that there are no conflicts of interest.

## Contributions

Conceived and designed the experiments: MM and MV. Performed the experiments: MFAB, SH, MB, MCO, CK, CD, MV and MM. Analyzed the data: MFAB, SH, MCO, MV and MM. Contributed reagents/materials/analysis tools: MV, CD, MFAB and MM. Wrote the paper: MFAB, MV and MM. Interpreted results of experiments: MFAB, SH, MV and MM. Prepared figures: MFAB and MM. Edited and revised the manuscript: MFAB, MCO, MV and MM.

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