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Reversing gene expression in cardiovascular target organs following chronic depression of the paraventricular nucleus and rostral ventrolateral medulla in spontaneous hypertensive rats

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ABSTRACT

Background: Arterial Hypertension (AHT) and its development are associated with structural, functional and genomic expression alterations in several organs, which all contribute to cardiovascular risk. In conditions when AHT is uncontrolled genomic expression can change, accelerate organ damage and ultimately results in organ failure. In previous studies, we have shown that chronic overexpression of an inwardly rectifying potassium channel (hKir2.1) in the paraventricular nucleus of the hypothalamus (PVN) and in the rostral ventrolateral medulla (RVLM), to suppress neuronal excitability, resulted in a long term decrease of blood pressure and sympathetic output in spontaneously hypertensive rats (SHR) but it remains unclear whether the pathological changes in gene expression relating to end organ structure are normalised.

Objective: To evaluate gene expression changes in tissues controlling blood pressure (the heart, kidney and common carotid artery) induced by chronic overexpression of hKir2.1 channels in either the PVN or RVLM.

Methods: We recently demonstrated in radio-telemetered spontaneously hypertensive rats (SHR) that overexpression of hKir2.1 in the PVN or RVLM decreased blood pressure and sympathetic output (−22±6 mmHg and -0.37±0.11 $mmHg^2ms^{-2}$ in PVN and -40±10 mmHg and -0.27±0.11 $mmHg^2ms^{-2}$ in RVLM). The mRNA levels of 13 pre-selected genes known to be involved with blood pressure regulation were evaluated using real-time polymerase chain reaction (RT-PCR) in tissue from the kidney, heart and common carotid artery of hKir2.1 SHR, sham SHR and normotensive rats.

Results: In hearts from SHR in which either the PVN or RVLM were injected with LVhKir2.1, there was a downregulation of angiotensin II receptor, type 1a (AT1), ATPase, Ca^{2+} transporter type 2C1 (ATP2C1) and troponin T type 2 (Tnnt2) relative to the sham group. Also, tropomyosin 2, beta (Tpm2) was down-regulated in hearts from SHR injected in RVLM. In the kidney of SHR with LVhKir2.1 injections in PVN and RVLM, angiotensinogen, angiotensin II receptor type 2 (AT2) and endothelin 1 (ET-1) were all upregulated compared to sham. In the carotid artery, endothelin 2 (ET-2) was up-regulated following LVhKir2.1 in to either the PVN or RVLM relative to sham.

Conclusion: Central modulation of PVN or RVLM neuronal excitability that promotes a decrease in blood pressure and sympathetic activity affected gene expression in three end-organs, mainly through the up-regulation of angiotensinogen and AT-2 genes in the kidney and down-regulation of AT-1 in the heart. These results provide new insights into the molecular mechanisms underlying the potential efficacy of chronic overexpression of hKir2.1 channels in central sympathoexcitatory areas in protecting against end-organ damage in essential hypertension and thus reveal peripheral targets for therapeutic manipulation in hypertension.

1. INTRODUCTION

Arterial Hypertension (AHT) and its development are associated with chronic activation of the sympathetic nervous system along with alterations in structural, functional and genomic expression in several organs, in particular in target organs such as the brain, heart, kidney and vasculature, all of which contribute to cardiovascular risk.

In conditions when AHT is uncontrolled, genomic expression and transcriptomic alterations can evoke changes in different signal-transducing cascades, thus, accelerating end-organ damage which ultimately results in organ failure and secondary disease such as stroke, cardiac ischemia and nephropathy (REFRENCES). An elevated blood pressure can also cause left ventricular hypertrophy, aortic stiffness, atherosclerotic plaques and microvascular disease that may render AHT more difficult to control (Muiesan ML, 2013; Cecelja M, 2012; Ausiello D, 2003).

In previous studies, we have shown that chronic overexpression of an inwardly rectifying potassium channel (hKir2.1) in the paraventricular nucleus of the hypothalamus (PVN) and in the rostral ventrolateral medulla (RVLM), to suppress neuronal excitability, resulted in a long term (8 weeks) decrease of blood pressure and sympathetic output to cardiovascular system in spontaneously hypertensive rats (SHR) but not in normotensive Wistar Kyoto (WKY) rats. However, it remains unclear whether the pathological changes in gene expression relating to end organ structure in the SHR can be normalised when arterial pressure and sympathetic tone are reduced by hKir2.1 overexpression in the PVN and RVLM. Thus, the present study has used quantitative real-time pcr analysis to assess whether genes expected to be altered in cardiovascular pathologies (e.g., renin-angiotensin system, nitric oxide metabolism and signalling, vasoconstrictiors and dilators) can be rescued following a chronic lowering of blood pressure and sympathetic activity centrally in the heart, kidney and common carotid artery) of the SHR.

2. MATERIALS AND METHODS

All the experimental procedures were in accordance with the European and Portuguese Law on animal welfare and had the approval of the ethic committee of the Faculty of Medicine, University of Lisbon, Portugal. WKY rats (n=7) and SHRs (n=18), males, aged 12 weeks and weighing 363±8g, were used. SHR rats were bilaterally microinjected with LV-hKir2.1 (LV-TREtight-Kir-cIRES-GFP 5.4x10E9 and LV-Syn-Eff-G4BS-Syn-Tetoff 6.2x10E9 in a ratio 1:4) into the PVN or RVLM. SHAM rats were bilaterally microinjected in the same regions with LV-eGFP

(LV-TREtight-GFP 5.7x10E9 and LV-Syn-Eff-G4BS-Syn-Tetoff 6.2x10E9 in a ratio 1:4) as shown in previous studies (Geraldes et al., 2014a; Geraldes et al, 2014b).

2.1. Organ tissue processing, RNA isolation and cDNA Synthesis

Animals were killed with an overdose of anesthesia (pentobarbital, 50 mg/kg, i.v.) and the target organs (whole heart, common carotid artery and whole kidney) were excised, immediately frozen separately in liquid nitrogen and stored at −80 ◦C for subsequent RNA isolation.

The heart, common carotid artery and kidney of individual LVhKir2.1 injected SHR, Sham SHR and WKY rats were grinded with a mortar and pestle in liquid nitrogen and approximately 50mg of powdered tissue was homogenized in Tri Reagent® Solution (Ambion) for total RNA isolation according to the manufacturer's instructions. RNA concentration was estimated by measuring the absorbance at 260nm and its purity assessed by determining the 260/280nm absorbance ratio using NanoDrop 1000A (Thermo Scientific). First strand cDNA was synthesized from 1µg of total RNA using High Capacity cDNA Reverse Transcription Kit (Applied
Biosystems), according to the manufacturer's instructions.

2.2. Quantitative real-time PCR analysis

Oligonucleotide primers were designed with Primer Express ® Software Version 3.0 (Applied Biosystems) according to the recommended parameters for quantitative assays, based on the mRNA sequences obtained from the Rat Genome Database. Gene ID and oligonucleotide sequences are listed in Table 1. The chosen genes are known to be involved in various processes including blood pressure regulation, renin-angiotensin system, nitric oxide metabolism and signalling, vasoconstriction/vasodilatation, osmotic shock, ion transport, nitric oxide metabolism, hypoxia response and vasotone.

RNA concentration was determined by measuring its absorbance in the NanoDrop 1000A (Thermo Scientific). The A260/A280 ratio of RNA 260/A280 ratios exceeded 1.9 showing minimal protein contamination.

Real-time PCR reactions were performed on a 7500 Fast Real-Time PCR System (Applied

Biosystems) using Fast SYBR® Green Master Mix reagents, following the manufacturer's protocol. Immediately after amplification, melt curve analysis was performed in order to check

PCR reactions for primer-dimer artifacts and to ensure specificity. To confirm accuracy and reproducibility of real-time PCR the intra-assay precision was determined in three repeats.

Table 1 - Primers and respective sequences designed for Real Time PCR

Gene name (Symbol) **Accession number**	**Primer sequence** **Forward/Reverse**
Angiotensinogen (Agt) NM_134432	CCCTGAGCAGTCCGTTCCT AAAGTGCAGCGCACCTGAGT
Angiotensin II receptor, type 1a (AT1a) NM_030985	GCCAGGGCAGCCTCTGA TCCTGAGGCAGGGTGAATG
Angiotensin II receptor, type 1b (AT1b) NM_ 112271	CCTCCGCCGCACGAT CCATTAGCCAGATGATGATGCA
Angiotensin II receptor, type 2 (AT2) NM_012494	TGCTGTTGTGTTGGCATTCA ATCCAAGAAGGTCAGAACATGGA
ATPase, Ca++ transporting, type 2C, member 1 (Atp2c1) NM_131907	TGGAACCCTGACGAAGAATGA GCATGCAGGCCGTCTGA
Endothelin 1 (ET-1) NM_012548	TGGAGGCCATCAGCAACAG AGTTCCGCTTTCAACTTTGCA
Nitric oxide synthase 3, endothelial cell (Nos3) NM_021838	TCTTTCGGAAGGCGTTTGAC CTCTAGGGATACCACATCGTATTCATC
Renin (Ren) NM_012642	CTGCTCAGGCTGTTGATGGA CACCCTCTGGGAGAGAATGTG
Troponin T type 2 (cardiac) (Tnnt2) NM_012676	CAGGAAGCGCATGGAGAAG TCGAAGTGAGCCTCGATCAGA
Tropomyosin 1, alpha (Tpm1) NM_019131	GGCCAAGCACATTGCTGAA GCTTACGGGCCACCTCTTC
Tropomyosin 2, beta (Tpm2) NM_001024345	TAACCTGTCCCGGGTGCAT GCGAGCGGTGAAGAGTAGGTA
myosin, heavy chain 6, cardiac muscle, alpha (Myh6) NM_017239	ACAGAGTGCTTCGTGCCTGAT CAGTCACCGTCTTGCCGTTT
myosin, heavy chain 7, cardiac muscle, beta (Myh7) NM_017240	CAGCCTACCTCATGGGACTGA TGACATACTCGTTGCCCACTTT
Actin, beta ActB/ NM_031144	ACCAGTTCGCCATGGATGAC TGCCGGAGCCGTTGTC

The relative quantification of gene expression in LVhKir2.1 injected SHR, sham SHR and WKY samples was determined by comparative C(t) method, using the normotensive WKY group as a calibrator to estimate the relative amount of mRNA in both SHR groups. The mRNA level of all samples was normalized against an endogenous control (β-actin). The fold-change for the SHR samples relative to WKY was calculated by $2^{-()}$, where ( ) = ( ) −

() ,and ()() − () .

2.3. Ventricular wall measurements

Thicknesses of the left and right ventricular wall were evaluated in WKY, sham SHR and LVhKir2.1 injected SHR rats. Thicknesses were determined with a microscope (Olympus CX41) set at a total magnification of ×5.

2.4. Statistical analysis

Comparisons between groups for the same period and also comparisons within the same group, before and after the microinjections were performed. For the statistical analysis,

Student's t test for paired data and ANOVA for comparisons between inter-groups were used. All data were expressed as mean±SEM and passed the normality test. Significance was taken as

$P < 0.05$.

3. RESULTS

3.1. Lentiviral effect on blood pressure and sympathetic output

We recently demonstrated in radio-telemetered spontaneously hypertensive rats (SHR) that the overexpression of hKir2.1 in the PVN or RVLM of conscious unrestrained SHR, caused a marked and sustained decrease in blood pressure and sympathetic output as revealed indirectly by a decrease in the power density of the Low frequency (LF) band of systolic blood pressure (−22±6 mmHg and -0.37±0.11 $mmHg^2ms^{-2}$ in PVN and -40±10 mmHg and -0.27±0.11 $mmHg^2ms^{-2}$ in RVLM) (Geraldes et al., 2014a; Geraldes et al, 2014b). At the same time, SHR SHAM group were showing increased values of blood pressure, consistent with their developmental trend.

3.2. Changes in gene expression in the heart, common carotid artery and kidney induced by LV-hKir2.1 treatment

The present study was designed to identify which genes known to be involved in end-organ damage. In hypertension are changed in expression by LV-hKir2.1 injection in either the PVN or RVLM in individual end-organs.

Using RT-PCR, the expression profile of 16 genes was analyzed in the heart, common carotid artery and kidneys in LVhKir2.1 injected SHR, Sham SHR and sham WKY rats. The genes quantified for each target organ are shown in Table 2. The results are shown as below separately for each gene, tissue and microinjected area (Figure 1 to 3; Table 3, 4 and 5). A summary of mRNA expression changes in the 3 end-organs of LVhKir2.1 injected PVN and RVLM is presented in figure 4.

Table 2 - Selected genes and samples analyzed

Gene	**Target Organ Sample**
angiotensinogen (Agt)	Kidney
angiotensin II receptor, type 1a (AT1a)	Kidney
angiotensin II receptor, type 1b (AT1b)	Kidney, Heart
angiotensin II receptor, type 2 (AT2)	Kidney
ATPase, Ca++ transporting, type 2C, member 1 (Atp2c1)	Kidney, Heart
endothelin 1 (ET-1)	Kidney, Heart, Carotid
endothelin 2 (ET-2)	Carotid
endothelin receptor type A (Ednra)	Carotid
endothelin receptor type B (Ednrb)	Carotid
nitric oxide synthase 3, endothelial cell (Nos3)	Kidney, Heart
renin (Ren)	Kidney
troponin T type 2 (cardiac) (Tnnt2)	Heart
tropomyosin 1, alpha (Tpm1)	Heart
tropomyosin 2, beta (Tpm2)	Heart
myosin, heavy chain 6, cardiac muscle, alpha (Myh6)	Heart
myosin, heavy chain 7, cardiac muscle, beta (Myh7)	Heart

actin, beta (Actb)	Kidney, Heart, Carotid

3.2.1. Changes in mRNA expression in the heart

mRNA expression in PVN LVhKir2.1 injected SHR was similar to WKY rats. In the RVLM LVhKir2.1 injected SHR among the 9 genes studied only 1 was down-regulated – myosin 7 - compared with WKY group (Figure 1, table 3).

Comparing the mRNA expression in LVhKir2.1 injected SHR with the SHR sham: AT1, ATP2C1 and Tnnt2 were down-regulated in LVhKir2.1 injected PVN SHR and in LVhKir2.1 injected RVLM SHR. Also, Tpm2 was down-regulated in LVhKir2.1 injected RVLM SHRs (table 3).

Table 3 - mRNAs Expression in the heart of SHR after the treatment with LV-hKir2.1 in the PVN and in the RVLM relative to WKY group or to SHR SHAM group. *p<0.05; **p<0.01.

PVN SHR Genes	Fold change relative to WKY	Fold change relative to SHAM	RVLM SHR Genes	Fold change relative to WKY	Fold change relative to SHAM
AT1	1,36	0,26*	**AT1**	1,38	0,26*
Atp2C1	0,74	0,38**	**Atp2C1**	0,78	0,40**
ET-1	1,25	2,05	**ET-1**	1,65	2,35
Myh6	0,62	0,85	**Myh6**	0,49	0,68
Myh7	0,43	0,76	**Myh7**	0,28*	0,72
NOS3	0,57	1,19	**NOS3**	0,98	2,05
Tnnt2	0,70	0,36*	**Tnnt2**	0,76	0,50*
Tpm1	0,78	0,43	**Tpm1**	0,77	0,42
Tpm2	1,34	0,99	**Tpm2**	1,08	0,34*

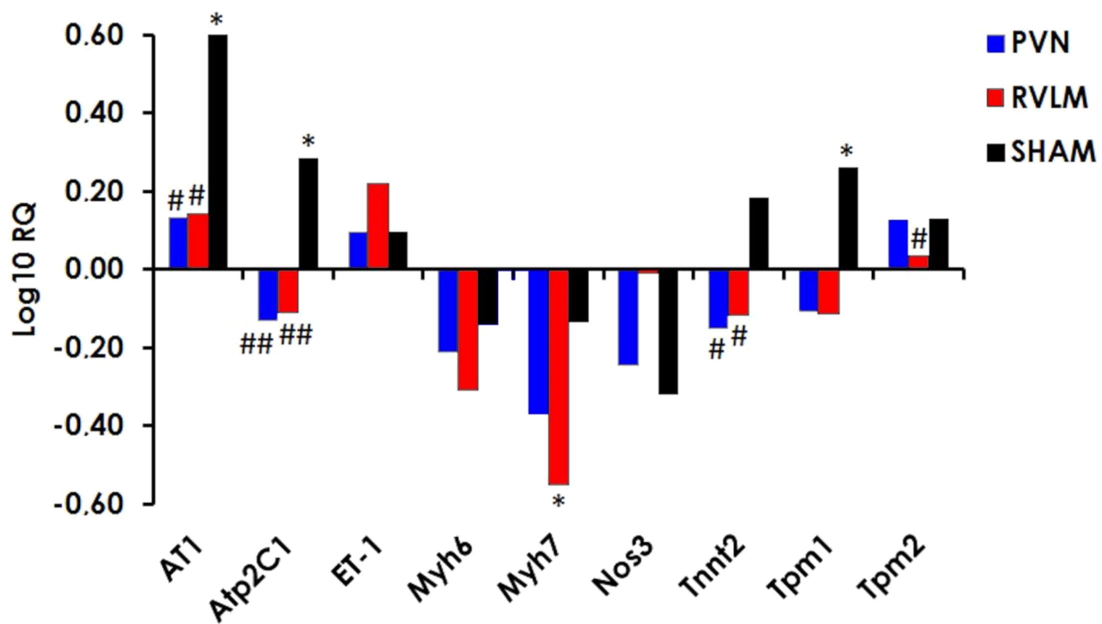

Figure 1 – mRNA expression in the heart of LVhKir2.1 injected PVN and RVLM SHR and SHR SHAM relative to WKY rats. AT1, angiotensin II receptor type 1; Atp2C1, ATPase, Ca++ transporting, type 2C, member 1; ET-1, endothelin 1; Myh6, Myosin 6; Myh7, Myosin 7; Nos3, Nitric oxide synthase 3, endothelial cell; Tnnt2, Troponin T type 2; Tpm1, Tropomyosin 1, alpha; Tpm2, Tropomyosin 2, beta. *p<0.05 compared to WKY rats and $^{\#}$p<0.05; $^{\#\#}$p<0.01 compared to SHAM rats.

3.2.2. Expression changes in the kidney

Comparing the mRNA expression in LVhKir2.1 injected SHR (PVN and RVLM) with WKY rats 2 of 8 genes were up-regulated – endothelin 1 and AT2, respectively (Figure 2, table 4).

Comparing the mRNA expression in LVhKir2.1 injected SHR with sham SHR rats: 3 genes were up-regulated – Angiotensinogen, AT2 and ET-1 in LVhKir2.1 injected PVN SHR and in LVhKir2.1 injected RVLM SHR, respectively (table 4).

Table 4 - mRNAs Expression in the kidney of SHR after the treatment with LV-hKir2.1 in the PVN and in the RVLM, relative to WKY group or to SHR SHAM group. *p<0.05; **p<0.01; ***p<0.001

PVN SHR Genes	Fold change relative to WKY	Fold change relative to SHAM	RVLM SHR Genes	Fold change relative to WKY	Fold change relative to SHAM
Agt	1,09	1,91**	**Agt**	1,51	2,67**
AT1a	1,10	0,80	**AT1a**	1,16	0,85

AT1b	0,85	0,59	AT1b	1,60	1,10
AT2	2,20*	11,10*	**AT2**	8,34*	42,10***
Atp2C1	1,20	1,55	**Atp2C1**	1,01	1,31
ET-1	3,06***	8,47***	**ET-1**	5,30***	14,69***
NOS3	0,62	0,50	**NOS3**	0,83	0,66
Ren	1,21	0,71	**Ren**	1,52	0,89

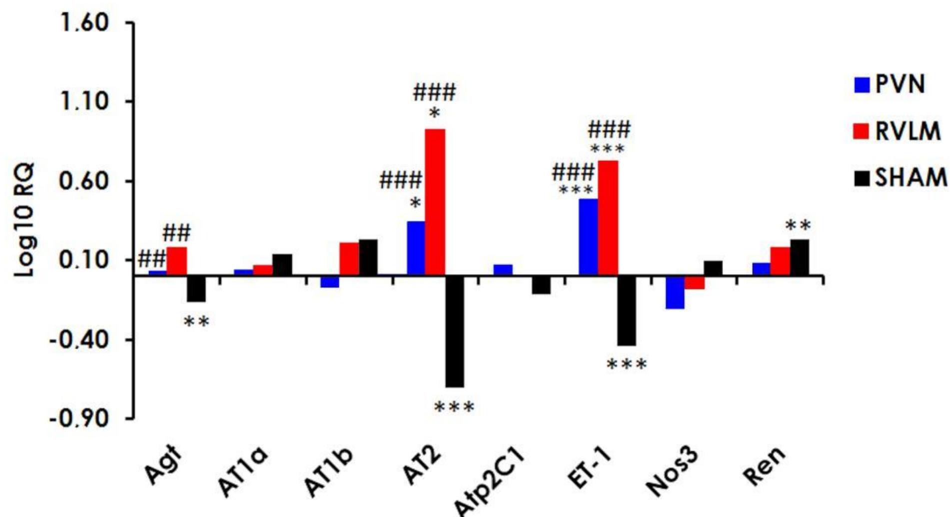

Figure 2 – mRNA expression in the kidney of LVhKir2.1 injected PVN and RVLM SHR and SHR SHAM relative to WKY rats. Agt, Angiotensinogen; AT1a, angiotensin II receptor type 1a; AT1b, angiotensin II receptor type 1b; AT2, angiotensin II receptor type 2; Atp2C1, ATPase, Ca++ transporting, type 2C, member 1; ET-1, endothelin 1; Nos3, Nitric oxide synthase 3, endothelial cell; Ren, Renin. *$p<0.05$; **$p<0.01$; ***$p<0.001$ compared to WKY rats and $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ compared to SHAM rats.

3.2.3. Expression changes in the common carotid artery

In LVhKir2.1 injected PVN SHR, among the 4 genes studied one was up-regulated - endothelin receptor type A (Ednra) – compared with WKY rats (Figure 3, Table 5). Comparing the mRNA expression in LVhKir2.1 injected PVN SHR with sham SHR rats, 1 gene was up-regulated - endothelin-2 (ET-2) (Figure 3, table 5).

In LVhKir2.1 injected RVLM SHR, 1 gene was up-regulated – endothelin receptor type A (Ednra) - compared with WKY rats (table 5). Compared to the sham SHR group there was an up-regulation of ET-2 in RVLM LVhKir2.1 injected SHR (table 5).

Table 5 - mRNAs Expression in the common carotid artery of SHR after the treatment with LV-hKir2.1 in the PVN and in the RVLM relative to the WKY group or to SHR SHAM group. *p<0.05

PVN SHR Genes	Fold change relative to WKY	Fold change relative to SHAM	RVLM SHR Genes	Fold change relative to WKY	Fold change relative to SHAM
ET-1	1,54	1,09	**ET-1**	1,38	0,97
ET-2	3,29	4,76*	**ET-2**	2,06	2,99*
Ednra	1,70*	2,55	**Ednra**	1,49*	1,75
Ednrb	2,26	2,94	**Ednrb**	1,75	2,27

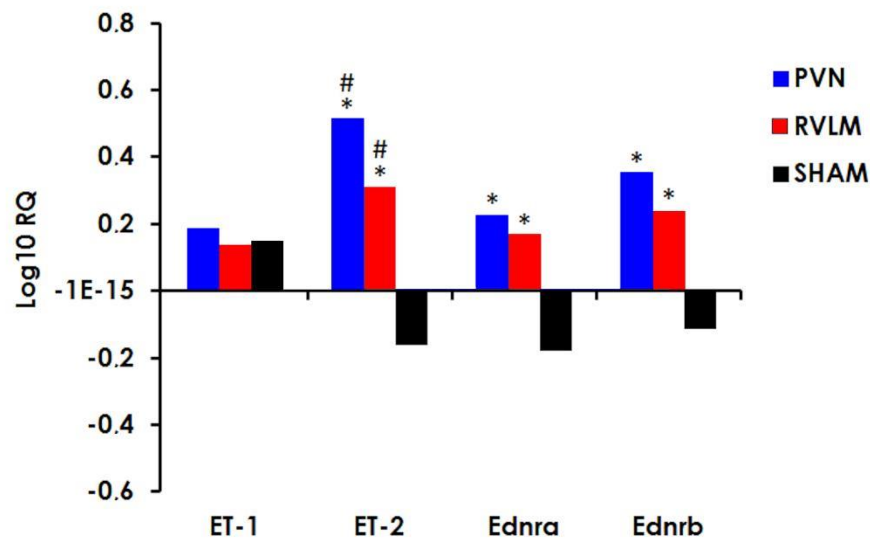

Figure 3 – mRNA expression in the common carotid artery of LVhKir2.1 injected PVN and RVLM SHR and SHR SHAM relative to WKY rats. ET-1, endothelin 1; ET-2, endothelin 2; Ednra, endothelin receptor type A; Ednrb, endothelin receptor type B. *p<0.05;***p<0.001.

3.3 Effects of the LV-hkir2.1 microinjection on ventricular (LV) wall thickness in SHR

Sham SHR animals had significantly elevated heart weight compared to WKY rats, with an increase of 11±3%. Also, the left ventricle thickness/right ventricle thickness ratio was increased significantly by 181±8% in sham SHRs compared with WKY rats ($p<0.05$). These data indicate, as expected, hypertrophy in SHRs.

The LVhKir2.1 injected SHR showed a reduction of heart weight (12±3%) compared with sham SHR rats. The left ventricle thickness/right ventricle thickness ratio was also decreased by 51±6% in LVhKir2.1 injected SHRs compared with sham SHR rats ($p<0.05$).

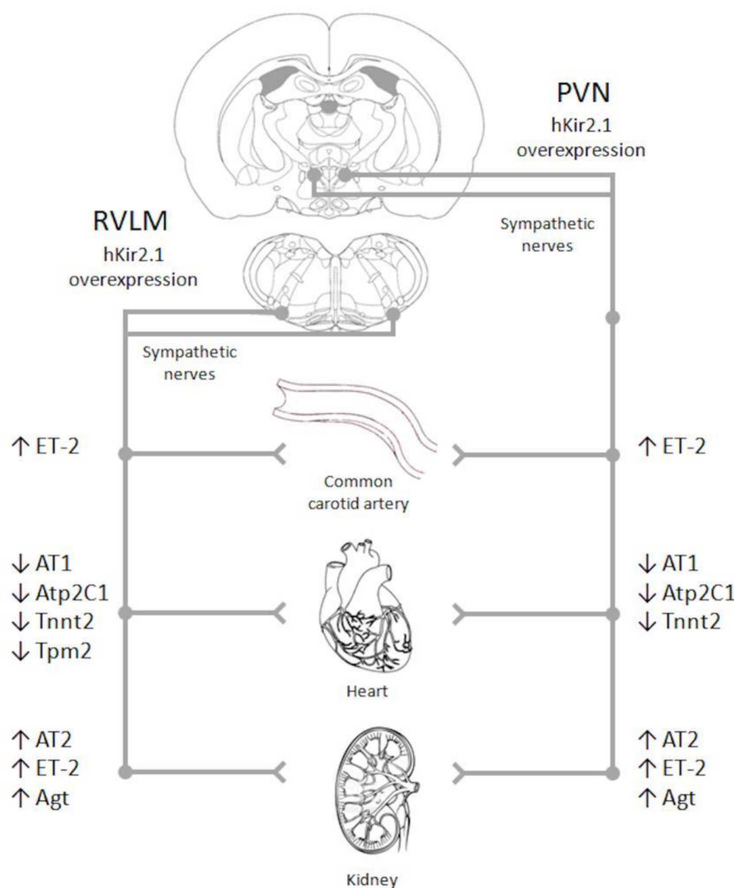

Figure 4 – Summary of mRNA expression changes in the common carotid artery, heart and kidney of LVhKir2.1 injected PVN and LVhKir2.1 injected RVLM compared to SHR SHAM.

4. DISCUSSION

We previously showed that a decrease in neuronal excitability within PVN and RVLM of SHR caused a chronically sustained decrease in blood pressure and sympathetic output (Duale *et al.*, 2005a, Duale *et al.*, 2005b, Geraldes *et al.*, 2013). In order to determine if the decrease in central neuronal excitability induced by the chronic overexpression of hKir2.1 channels in the PVN and RVLM reversed pathological remodeling in target organs, we evaluated gene

expression changes in the heart, common carotid artery and kidneys, some of the major end-organs associated with arterial hypertension (HTA).

Our main finding of the present study was that treatment of LV-hKir2.1 in the PVN and RVLM of SHR can promote a remodeling process in target organs, as shown by the different regulation of gene expression in cardiac, common carotid artery and renal tissues.

a) Heart and common carotid artery

Cardiac hypertrophy is one of the major hypertension-induced pathological consequences. Taking in to account the 9 genes evaluated in the present study, only 1 was different from WKY rats, namely the myosin heavy chain β (β-MHC or Myh7) was downregulated in LVhKir2.1 injected RVLM SHR. β-MHC 7 is involved in the regulation of contractility or hypertrophy. In normal cardiac tissue the myosin heavy chain α (α-MHC; v1 cardiac myosin) is expressed predominantly, but in models of cardiac hypertrophy, the β-MHC has a higher expression than the α-MHC (Mercadier *et al.*, 1981).

Compagno showed that the β-MHC was markedly expressed in the ventricle of 15-week old SHR vs WKY (Compagno *et al.*, 2001). Another study showed that after angiotensin II type 1 (AT1) receptor antagonist treatment, SHR showed BP decrease and downregulation of myosin heavy chain isoforms in smooth muscle cells of the aortic tunica media, that is similar to what happened to the Myh7 gene in the heart of LVhKir2.1 rats in the present study (Fujii *et al.*, 1999).

There was no significant difference in the expression of AT1 receptor, ATPase Ca^{2+}(ATP2C1), Endothelin-1, Myosin 6, Nitric oxide synthase 3, Troponin T2 (Tpm2), Tropomyosin 1 (α) and Tropomyosin 2 (β) in the heart following LVhKir2.1 injections in PVN and RVLM between SHR and WKY rats. Previous studies showed differences in the expression of these genes in SHR vs WKY rats indicating remodeling processes in the heart of LVhKir2.1 injected SHR. In fact, the AT1 receptor is up-regulated and there is evidence indicating a decrease in ATPase Ca^{2+} expression is prominent in hypertrophy and in heart failure, however it was shown that the expression of SR ATPase Ca^{2+} was not down-regulated in the heart of 11 week old SHR (Ohta *et al.*, 1995). In addition, consistent with our findings in SHAM animals, other studies showed that troponin is up-regulated and NO synthase is downregulated in the heart of SHR in comparison with the WKY rats (Bauersachs *et al.*, 1998; Piech *et al.*, 2003). LVhKir2.1 microinjection in PVN or RVLM promoted a significant troponin down-regulation when compared to SHAM SHR.

In the heart of LVhKir2.1 injected PVN or RVLM we also observed a down-regulation of AT1 receptors and ATP2C1 when compared to the SHR SHAM group. The RVLM group has also

showed down-regulation of Tpm2. Thus, it seems that there is a remodeling process in the hypertrophied heart of this animal model, since there is a continued improvement in gene expression in the heart of these animals, approaching normal levels after LVhKir2.1 treatment in PVN or RVLM. Moreover we show that these changes were correlated with left ventricular wall thickness, since the chronic treatment with LV-hKir2.1 in SHR induced regression of this tissue.

In the common carotid artery there was an up-regulation of endothelin-2 (ET-2), endothelin receptor type A (Ednra) and type B (Ednrb) in LVhKir2.1 injected PVN and RVLM SHR in comparison to WKY rats. When compared to the sham group, the LVhKir2.1 injected PVN and RVLM SHR showed an up-regulation of ET-2. Interestingly, not all hypertensive conditions have elevated endothelin levels. It is well established that endothelin-1 (ET-1) gene expression in blood vessels, such aorta and mesenteric arteries of adult SHR are normal or reduced compared to normotensive WKY rats (Schiffrin EL, 1995; Larivière R, 1995). This is in agreement with our results, since ET-1 gene expression in the common carotid artery in all SHR was similar to WKY rats.

Although ET-2 is pharmacologically indistinguishable from ET-1, we found an increase in ET-2 gene expression in LVhKir2.1 injected SHR and ET-2 expression did not differ significantly between WKY and sham SHR. In a transgenic rat overexpressing ET-2 there is evidence that ET-2 had no effect on the hypertensive condition (Paul M, 1994; Hocher B, 1996). Therefore, given the lack of knowledge about the biological function of endogenous ET-2, particularly in SHR model, it is difficult to speculate on the functional consequences of the increased ET-2 expression levels found in common carotid artery of the SHR.

The biological effects of endothelins are mediated by two receptors named Ednra and Ednrb receptors. In the vasculature, Ednra receptor mediates vasoconstriction (Haynes & Webb, 1993; Janakidevi et al., 1992) while the Ednrb receptors mediate vasodilatation through the release of nitric oxide and/or prostacyclin (Hirata et al., 1993; Filep et al., 1991; Schneider et al., 2007). Therefore, in our study, the increased expression of Ednrb receptors may improve blood flow through their known vasodilator function.

b) Kidney

In the kidneys of PVN and RVLM LVhKir2.1 injected SHR 2 of 8 genes studied were up-regulated (ET-1 and AT2) when compared to WKY rats. ET-1 is a potent vasoconstrictor but the role of ET-1 in SHR remains unclear. Hughes et al found that SHR and aged-matched WKY rats had no difference in renal ET-1 levels until arterial hypertension (AHT) appeared. After the

development of AHT the SHR had a significantly reduced ET-1 in the urine and in the outer and inner medulla of the kidney (Hughes *et al.*, 1992; Largo *et al.*, 1997). Hence, the up-regulation of ET-1 found in the kidneys of LVhKir2.1 injected SHR relative to normotensive rats (WKY) and to Sham SHR may not necessarily participate in the progression of AHT in this animal model.

Wu and colleagues observed in kidneys of SHR an up-regulation in Ang II receptor AT-1 in comparison to WKY rats. Our data show that the LV-treatment in SHR can reverses the mRNA levels of AT-1 in the kidneys, since these are similar to the mRNA AT-1 levels found in WKY rats. This would be predicted to limit the vasoconstrictive effect of Ang II. Our results also showed an up-regulation in AT-2 receptor in the LVhKir2.1 injected SHR, thus contradicting the biological effects of AT1 receptor activation, promoting vasodilation (Oparil *et al.*, 2003). Cosentino *et al.* showed that long-term treatment with the AT1R antagonist, losartan, in SHR promotes an increase in AT2R mRNA in thoracic aortas, supporting our finding about the beneficial up-regulation of AT2R mRNA in the kidney of LVhKir2.1 injected SHR (Cosentino *et al.*, 2005).

According to several studies, there is an increase in renin mRNA expression in the kidneys of SHR (Antonaccio *et al.*, 1984; Samani *et al.*, 1989; Nakamura & Johns, 1995). Since renin release is regulated by the renal sympathetic nerves in the kidney we suggest that the decrease in sympathetic activity promoted by the LV-treatment is responsible, at least in part, for the decrease in renin expression found in the kidneys of LVhKir2.1 injected SHR (Skøtt & Jensen, 1993).

There is a positive correlation between the angiotensinogen levels and the blood pressure found in rats and in humans (Dzau VJ, 1989, El-Dahr SS, 1991, Page WV, 1992, Nakamura A, 1994; Bruna RD, 1993). The angiotensinogen-deficient mice do not produce angiotensinogen and are hypotensive reflecting the importance of angiotensinogen in the maintenance of BP and in the development of AHT (Pratt *et al.*, 1989; Tanimoto *et al.*, 1994).

However, the kidneys of SHR contain lower levels of angiotensinogen mRNA compared with the WKY rats (Pratt *et al.*, 1989). There is also evidence that low levels of renal sympathetic activity may increase angiotensinogen gene expression (Nakamura & Johns, 1994). In our study, the LV-treatment increased the angiotensinogen mRNA in the kidneys of LVhKir2.1 injected PVN and RVLM SHR compared to sham SHR and to similar levels found in WKY rats an effect that may be related to the decreased sympathetic activity.

We propose that a decrease of both blood pressure and sympathetic output contribute to the process of end-organ remodeling in the hypertensive state. The beneficial effect of

sympathetic downregulation and/or blood pressure decrease on cardiac structure and renal function is well established (Amann K, 2011; Weinberg E, 1997; Bautista R, 2001; Li Y, 2010). However, studies using a peripheral vasodilator that only lowers blood pressure are needed to understand if our hypothesis is correct.

In conclusion, central manipulation of sympathoexcitatory regions in the brain that promote a decrease in the blood pressure and sympathetic activity can affected mRNA expression in catdiovascular target organs, mainly through the up-regulation of angiotensinogen and AT-2 genes in the kidney and down-regulation of AT-1 in the heart. Our data reveal the organs and genes that could be specifically targeted to offset the pathological consequences of hypertension.

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