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ORIGINAL ARTICLE

Changes in expression of orphan receptors GPR99 and GPR107 during the development and establishment of hypertension in spontaneously hypertensive rats

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ABSTRACT

Hypertension is a disease, which in spite of existing treatments continues to have high morbidity and mortality, which suggests that there are other mechanisms involved in this pathology. In this sense, the orphan receptors are G protein-coupled receptor associated with various pathologies such as GPR99 which has been linked to mice develop left ventricular hypertrophy induced by blood pressure overload while GPR107 with patients with idiopathic pulmonary arterial hypertension. For this reason, the aim of this work was to study if the expression of the orphan receptors GPR99 and GPR107 are modified by arterial hypertension. Male SHR and WKY rats of 6–8 and 10–12 weeks old were used. The weight, systolic blood pressure and heart rate were measured, as well as the mRNA of the receptors GPR99 and GPR107 in the aorta, kidney, heart and brain by RT-PCR, also was realized an in silico analysis to predict which G protein could be coupled the orphan receptor GPR107. Our results showed that receptors GPR99 and GPR107 are expressed in the analyzed tissues and their expression profile tends to change at different ages and with the development of hypertension, for the other hand, the bioinformatics analysis for GPR107 showed that is coupled to Gi protein. Therefore, we do not rule out that GPR99 and GPR107 could be involved in the pathophysiology of hypertension and could be used as targets therapeutic in hypertension.

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Hypertension; GPR99; GPR107; orphan receptors; gene expression

Introduction

High blood pressure (HBP) is defined as a blood pressure greater than or equal to 130/80 mm Hg systolic (SBP) and diastolic blood pressure (DBP), respectively [1]. This disease is one of the main causes of mortality worldwide [2], it is estimated that its prevalence is approximately 1.39 billion and cause around 7.5 million deaths per year [3]. It is important to consider that despite the existence of different treatments for this pathology; it still has a high rate of morbidity and mortality, which suggests that there may be other mechanisms involved in the development of this disease. In this sense, a new class of G protein-coupled receptors (GPCRs), known as orphan receptors has been reported, so named because their endogenous ligand is currently unknown [4]. Orphan receptors have been classified according to the receptor that they have homology, being those of class A the largest family, which presents homology with rhodopsin receptors [5], class B with secretin [6], class C with metabotropic glutamate receptors [7] and last, are those of seven other transmembrane domains (7TM). These orphan receptors have been associated with various pathologies such as diabetes, hypertension, metabolic syndrome, cancer, etc. Some studies have been reported that their expression levels tend to increase or decrease, these changes have been associated not only in the development but also to the complications associated with the disease [8–11]. Among receptors that modify their expression during a pathological process are GPR99 and GPR107 receptors [12,13].

The GPR99 receptor is also known as GPR80, OXGR1, P2Y15 and α-KGR [14]. It is a desorphanized receptor and its endogenous ligand is an intermediary of the Krebs cycle (α-ketoglutarate) [14]. This receptor belongs to class A receptors of seven transmembrane domains and has been reported to be located in the chromosomal region 13q32.1 in humans, whereas in rats it is located on the chromosome 15q24, the homology of these receptors between both species are 86% and translate a protein of 337 amino acids [15]. Previous studies have identified that GPR99 is expressed in brain, kidney and heart. Interesting when this receptor is knocked out, it has been observed that mice develop left ventricular hypertrophy induced by blood pressure overload

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[14,16,17]. Therefore, we suppose that an alteration in GPR99 expression could develop arterial hypertension.

On the other hand, the orphan receptor GPR107 belongs to another class of 7TM receptors and is a member of the LUSTR family (lung seven-transmembrane receptor) because it has an extracellular domain highly conserved with glycine and arginine residues in its structure, it also has a structure of seven transmembrane domains common in all GPCRs [18]. It has been identified that this receptor is expressed in rats in tissues such as kidney and heart [19,20]. This receptor is located in chromosomal position 9q34.11 in humans, while in rats it is located in the 3p12 region; it also has a homology of 83% in its amino acid sequence between both species and encoding to protein with 600 amino acids in *Homo sapiens* and 551 in *Rattus norvegicus* [22]. It is not yet known which endogenous ligand binds to the orphan receptor GPR107 and for this reason, the function of this receptor is also unknown. However, there is evidence that the administration of neuronostatin induces the early expression of this receptor and increases blood pressure in rats. Interestingly, overexpression of GPR107 has been reported in patients with idiopathic pulmonary arterial hypertension, thus we do not rule out that GPR107 could be involved in hypertension [19,21]. Therefore, the evidence suggests that GPR99 and GPR107 receptors could be involved in the regulation of blood pressure and alterations in the expression of these two receptors could be associated with the development of hypertension. For this reason, the aim of this work was to evaluate whether GPR99 and GPR107 receptors participate in the development or establishment of hypertension.

Materials and methods

Animals

We used young male SHR and WKY rats (6–8 weeks old) and adults (10–12 weeks old). The animals were kept under normal conditions of 12 light/dark hours with rat feed and *ad libitum* water. The procedures used were approved by the Graduate Bioethics Committee of our institution with the registration number CICUAL-07/17-12-2014 and the Official Mexican Standard (NOM-062-ZOO 1999) concerning the techniques and production specifications, care and use of laboratory animals.

Physiological parameters

Weights of the rats were measured individually for each rat and in triplicate using a precision scale (Ohaus Scout pro CS200). The systolic blood pressure (SBP) and heart rate (HR) were measured by a noninvasive method in the rat́s tail (tail-cuff method), to develop this measurement the rats were placed in tubular acrylic cylinders (restrainers) and subsequently, a bracelet with a pressure sensor was placed in the rat́s tail. SBP and HR values were recorded in grass instruments polygraph model 79 (Quincy, Massachusetts, USA) and were averaged from at least three consecutive readings obtained from each rat.

Total RNA extraction

For the extraction of total RNA Trizol was used following the protocol described by the manufacturer (Invitrogen, Carlsbad, CA), then digestion of the genomic DNA was performed using a DNase (Roche, 04 716 728 001). The concentration and purity of the RNA were determined with a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington DE, USA). The samples fulfilled the ratio of 1.8 to 2.0 in 260/280 and 2.0–2.2 in 260/230. The RNA solutions were diluted to a working concentration of 1 μg/μl.

Quantification of mRNA expression of orphan receptors

For the synthesis of the cDNA, the system's reverse transcription kit was used (Promega Corporation, Woods Hollow Road, Madison, WI) using oligodT primers and following the manufacturer's recommendations. The retrotranscription reaction was incubated for 5 min at 25 °C, 60 min at 42 °C and 15 min at 70 °C. The qRT-PCR was performed with the SuperScript III Platinum One-Step RT-PCR System with Platinum® Taq DNA Polymerase kit under the manufacturer's instructions (Invitrogen, USA) and each sample was tested in triplicate. The Universal ProbeLibrary Assay Design Center [24] online program for the design of primers and probes was used. It introduced the FASTA sequence for GPR99 (NM_207588.1) and GPR107 (NM_001107828.1) available in the NCBI database [25]. We performed the polymerase chain reaction in a CFX-96 real-time thermal cycler (Bio-Rad, Hercules, CA, USA) using 96-well microplates under the following conditions: 95 °C for 2 min; 45 cycles at 95 °C for 15 s, 60 °C for 30 s. For amplification, the following primers and probes were used: GPR99 TGGCTTCCATTTCAACCTTT (Forward), CGGAAGAGGCTAAAGCAGGT (Reverse) and universal probe #89. GPR107 CAGGGCTTTCCGATTGAA (Forward), AGCAGTGCA CCCTTCAGC (Reverse) and universal probe #26. We used constitutive gene Hprt1 for normalization of mRNA data GGTCCATTCCTATGACTGTAGATTTT (Forward), AACAATCAAGA CGTTCTTTCCAG (Reverse) and probe #22. The negative control was obtained by performing real-time RT-PCR from two ways: 1) without RT for each cDNA synthesis and 2) without cDNA template for the reaction. The mRNA expression levels of GPR99 and GPR107 receptors were determined by the Livak method commonly known as 'delta delta Ct' and the analysis of the relative expression was normalized with the constitutive gene Hprt1 using the CFX96 software.

Prediction of the G protein subtype associated with the receptor

The bioinformatics analysis was carried out using the PRED PAR 2.00 online program (http://athina.biol.uoa.gr/bioinformatics/PRED-COUPLE2/) which considers the hidden Markov models (MOM) to classify the G-protein coupled receptors (GPCRs) using the sequence of conserved amino acids and also uses an artificial neural network to eliminate promiscuous couplings with coupling probability values below 0.3 [22].

Table 1. Physiological parameters for SHR and WKY at 6–8 and 10–12 weeks of age.

Groups	N	BW	SBP	HR
WKY 6–8 weeks–old	4	161 ± 4.86	106 ± 8.75	315 ± 37.75
SHR 6–8 weeks–old (Pre–hypertensive)	4	167 ± 4.51	120 ± 4.41	440 ± 28.28*
WKY 10–12 weeks–old	4	293 ± 10.68	116 ± 1.44	375 ± 15.00
SHR 10–12 weeks–old (Hypertensive)	4	257 ± 6.02*	174 ± 2.40*	405 ± 57.45

BW: Body weight; SBP: Systolic blood presuare; HR: Heart rate. Values are expressed as mean ± standard error of the mean (SEM) from SHR 6–8 weeks-old and 10–12 weeks-old compared age-matched WKY control. Data was statistically significant as $^{*}p \leq .05$ vs controls.

Statistical analysis

The data were reported as mean ± standard error of the mean (SEM), for the comparisons we used t-student and one-way ANOVA with a Tukey *post hoc* test. Significant values of $^{*}p \leq .05$, $^{**}p \leq .01$ and $^{***}p \leq .001$ were considered.

Results

Physiological parameters

The results showed that the SHR rats did not present changes in weight and blood pressure of 6–8 weeks old with compared to their WKY control. However, they present a significant increase in their heart rate concerning their control, while at 10–12 weeks the SHR rats showed a decrease in weight and a significant increase in blood pressure with respect to their WKY control (Table 1).

Expression of the desorphanized receptor GPR99

The expression analysis of receptor GPR99 in SHR rats of 6–8 weeks old showed a significant decrease in the left atrium with respect to its WKY control of the same age (Figure 2(C)), while in the aorta, kidney, brain, right atrium and ventricles no significant differences were observed (Figures 1(A–C) and 2(A,B,D)). The SHR rats of 10–12 weeks showed a significant increase in the aorta and right atrium (Figures 1(A) and 2(A)) while, the rest of the tissues were unchanged with respect to the WKY (Figures 1(B,C) and 2(B,C)), on the other hand, only aorta and right ventricle showed a higher expression Figures 1(A) and 2(A)) with respect SHR rats of 6–8 weeks old.

Expression of the orphan receptor GPR107

The expression analysis of receptor GPR107 showed in SHR rats of 6–8 weeks a significant increase in the aorta (Figure 3(A)) and a decrease in the right ventricle (Figure 4(C)). However, the rest of the analyzed tissues did not show significant differences in comparison with its WKY control (Figures 3(B,C) and 4(A,B,D)). On the other hand, the expression of this receptor in SHR rats of 10–12 weeks showed a significant increase in the right atrium and ventricle in contrast to their WKY control of the same age (Figure 4(A,C)), while in aorta, kidney, brain, atrium and left ventricle no significant changes were observed (Figures 3(A–C) and 4(B,D)). The results also showed that SHR rats of 10–12 weeks old compared with SHR of 6–8 weeks old showed a significant increase in the right atrium and ventricle (Figure 4(A,C)), whereas in aorta and brain the expression decreased (Figure 3(A,C)).

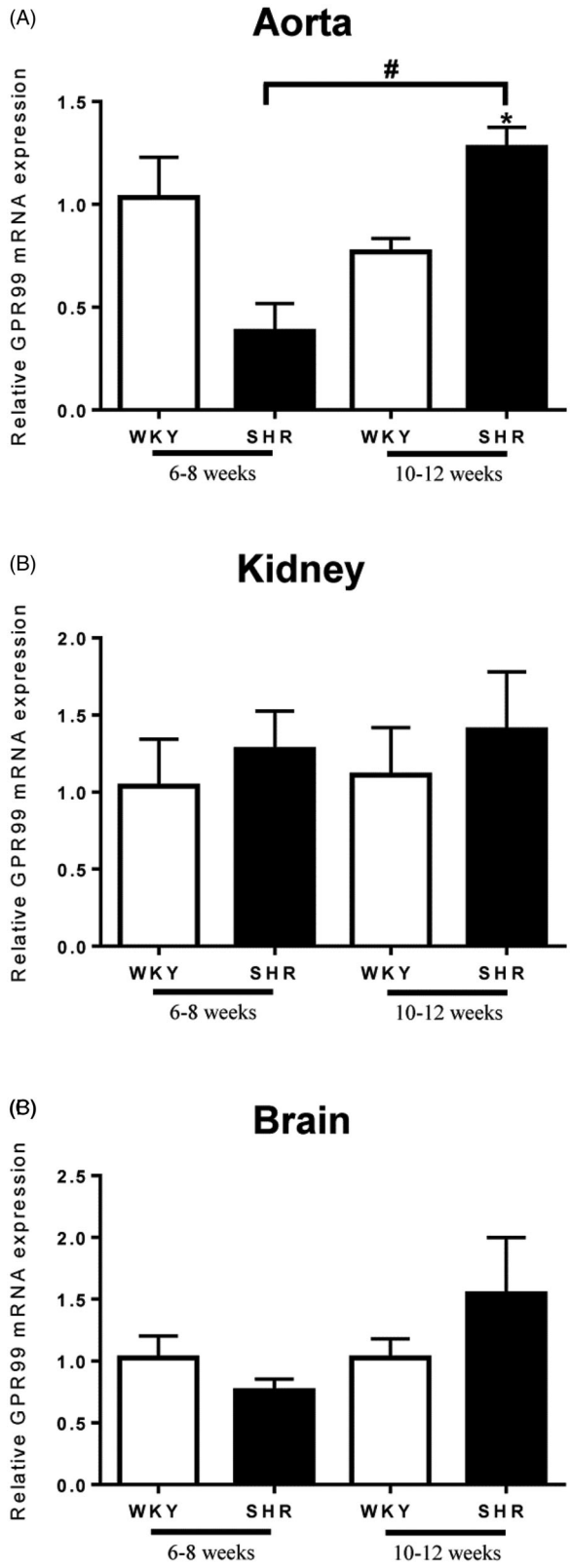

Figure 1. Relative expression of the orphan receptor GPR99 in (A) aorta, (B) kidney and (C) brain of rats with 6–8 and 10–12 weeks of age. Values are expressed as mean ± standard error. $^{*}p \leq .05$ vs WKY, $\#p \leq 005$ vs rats 6–8 weeks of each strain.

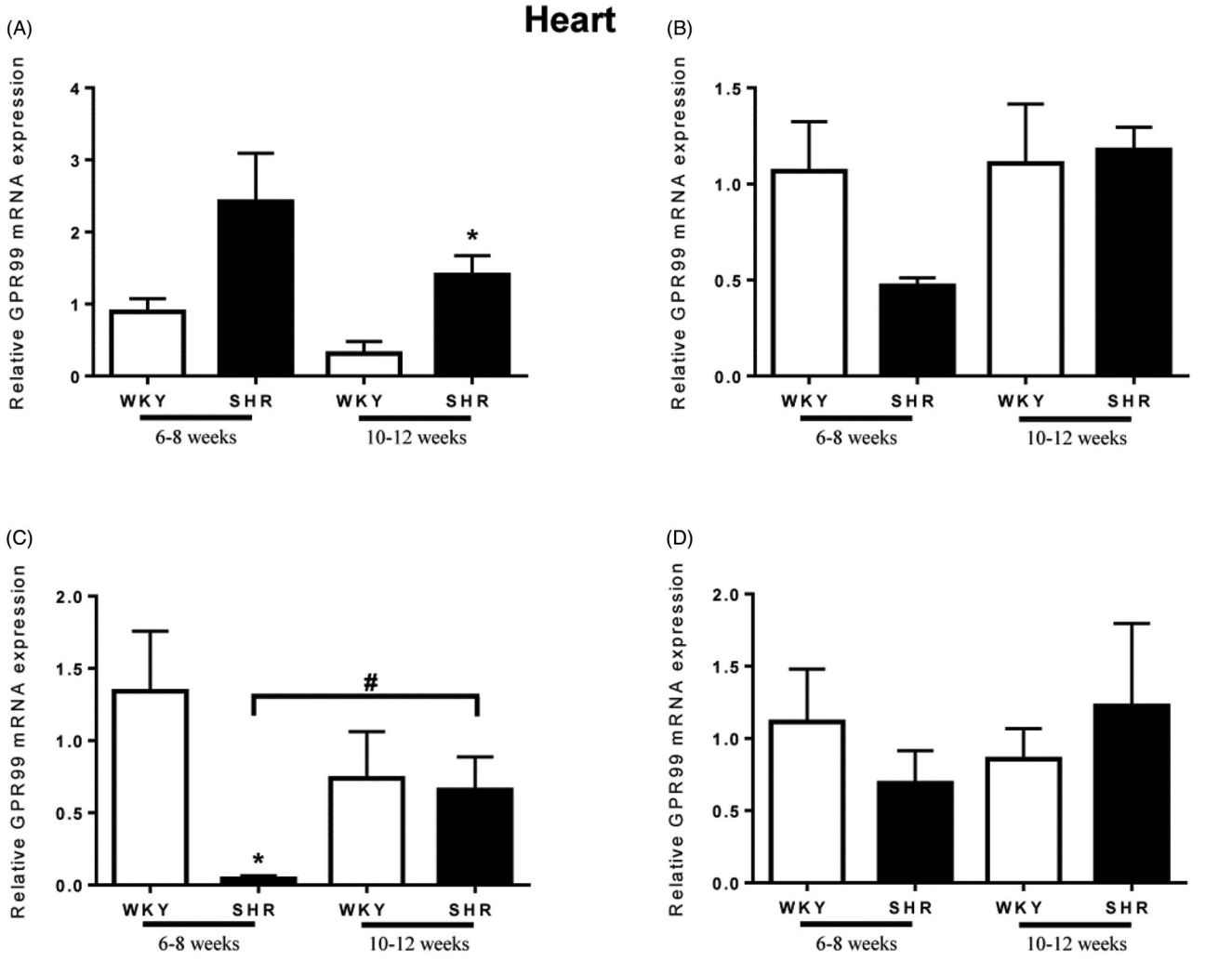

Figure 2. Relative expression of the orphan receptor GPR99 in (A) right atrium, (B) left atrium, (C) right ventricle and (D) left ventricle of rats with 6–8 and 10–12 weeks of age. Values are expressed as mean ± standard error. $^{*}p \leq .05$ vs WKY, $\#p \leq .05$ vs rats 6–8 weeks of each strain.

In silico prediction of the G protein that activates the orphan receptor GPR107

The bioinformatics analysis showed that the receptor has a high coupling probability to G proteins. GPR107 the putative G protein is a Gi/o protein, because to its predictions has a probability value greater than 0.9 was observed, which indicates that a value of 1 has a high probability that the receptor couple to that G protein, while a 0.3 value indicates low probability for the receptor to couple to the G protein.

Discussion

Our results showed that the adult SHRs presented lower weights, this agrees with previous studies where they associate this effect with a sympathetic hyperactivity that is observed in the SHR and maybe this effect causes an increase in the lipolysis [23,24] and significant decrease in the accumulation of triglyceride-rich lipoproteins in adipose tissue, in addition to activating β3 adrenergic receptors that stimulate thermogenesis by increasing energy expenditure [25]. It is important to mention no differences were observed in the weight of both strains of 6–8 weeks because at that age the SHR and WKY rats do not present differences in systolic blood pressure, suggesting that at that age they have not initiated sympathetic hyperactivity yet [26,27].

About the results of the systolic blood pressure, the SHR rats of 6–8 weeks compared with their control did not show differences, this is because the SHR rats at this age are considered pre-hypertensive [28,29]. However, as age advances, changes occur in the SHR strain that leads to the establishment of hypertension [30,31]. Hypertension in the SHR rat has been associated with an increase in peripheral vascular resistance [32] and sympathetic activity [33,34], as well as a decrease in vasodilators such as nitric oxide and prostacyclin [35,36].

In contrast, the heart rate only showed a significant increase in the SHR rats of 6–8 weeks, this result is in agreement with other studies where they report that the heart rate has a tendency to increase, but the blood pressure does not increase at that age because the vasculature retains its ability to be a counterbalance to avoid the increase in blood pressure; however, as age advances in the SHR strain, this counterbalance is lost and heart rate and blood pressure increase [37,38]. With regard to the expression of GPR99 and GPR107 receptors, these were expressed in all tissues

analyzed, but present differences in the expression profile between them, which agrees with the database of human genes (GeneCards) where it has been reported that the GPR99 receptor is expressed in several tissues including brain, heart and kidney [43].

The expression analysis for GPR99 receptor showed a localization and expression in kidney and brain of WKY and SHR rats of both ages, which coincides with previous works that report the expression of this receptor in these two tissues in mice [14,16,39]. However there is no evidence to show the expression of receptor GPR99 in young and adult WKY and SHR rats. The expression of GPR99 in aorta shows this receptor tends to increase its expression in SHR rats of 10–12 weeks, that is, it increases its expression in SHR adult rats with established hypertension. The GPR99 is an orphan receptor previously desorphanized by He and coworkers [14], who found that the activation of GPR99 by alpha-ketoglutarate stimulates the formation of inositol triphosphate (IP3) and increases the calcium flow in a dependent manner, therefore, its signaling pathway through of Gq protein coupling. The Gq protein-coupled receptors, such as GPR99, initiate the formation of inositol 1,4,5 trisphosphate (IP3) and diacylglycerol (DAG) that increase intracellular $Ca2^{+}$, causing vasoconstriction [40]. It has been reported that the signaling through Gq in vascular smooth muscle participates in two different murine models of hypertension. Therefore, we observed in this study that gpr99 expression increases in the aorta and consequently leads to an increase in Phospholipase C (PLC) activity and promote the calcium accumulation by sarcoplasmic reticulum causing vasoconstriction, in which case this mechanism could participate in the pathogenesis of hypertension. Hence, GPR99 receptor coupled to Gq protein could be playing a fundamental role in the vasoconstriction raising blood pressure favoring the establishment of hypertension. These findings coincide with our observations in the aorta where we found that the levels of expression for GPR99 in SHR rats of 6–8 weeks were diminished with respect to their control of the same age, which suggest a reduce activation of Gq protein, and as a consequence promote vasodilation. Therefore, this implies that low expression of GPR99 receptor could be involved in the control of blood pressure in SHR rats of 6–8 weeks, whereas that increase expression of GPR99 in SHR 10–12 weeks could be causing high blood pressure.

On the other hand, we also analyzed the expression of GPR99 in heart and found a reduction in the mRNA expression levels of this receptor in the right ventricle of pre-hypertensive SHR rats. This behavior suggests that GPR99 being a Gq protein-coupled receptor it could be involved in a cardioprotective effect in pre-hypertensive SHR rats due to the reduction of Gq activity, promoting the decrease of intracellular calcium in the sarcoplasmic reticulum causing negative chronotropism and inotropism [41]. Conversely, the expression of GPR99 increases in the aorta and right atrium in SHR rats of 10–12 weeks when the strain has already established hypertension causing an increase in the activity of the Gq protein and with it a positive chronotropism and inotropy [42]. Hence, this behavior suggests that GPR99 coupled to

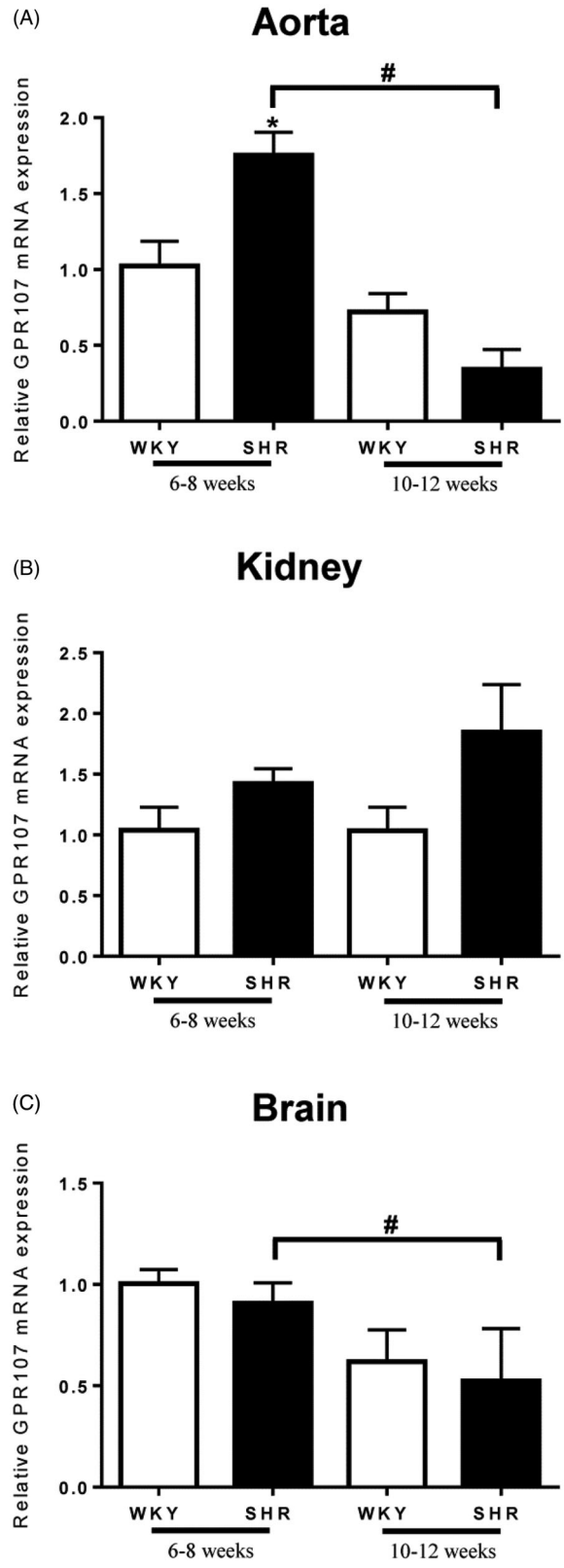

Figure 3. Relative expression of the orphan receptor GPR107 in (A) aorta, (B) kidney and (C) brain of rats with 6–8 and 10–12 weeks of age. Values are expressed as mean ± standard error. $^{*}p \leq .05$ vs WKY, $\#p \leq .05$ vs rats 6–8 weeks of each strain.

Gq protein could play an important role at a cardiovascular level in the pathogenesis of hypertension. There are previous studies that have reported that activation through Gq protein-coupled receptors, such as GPR99 in heart is associated

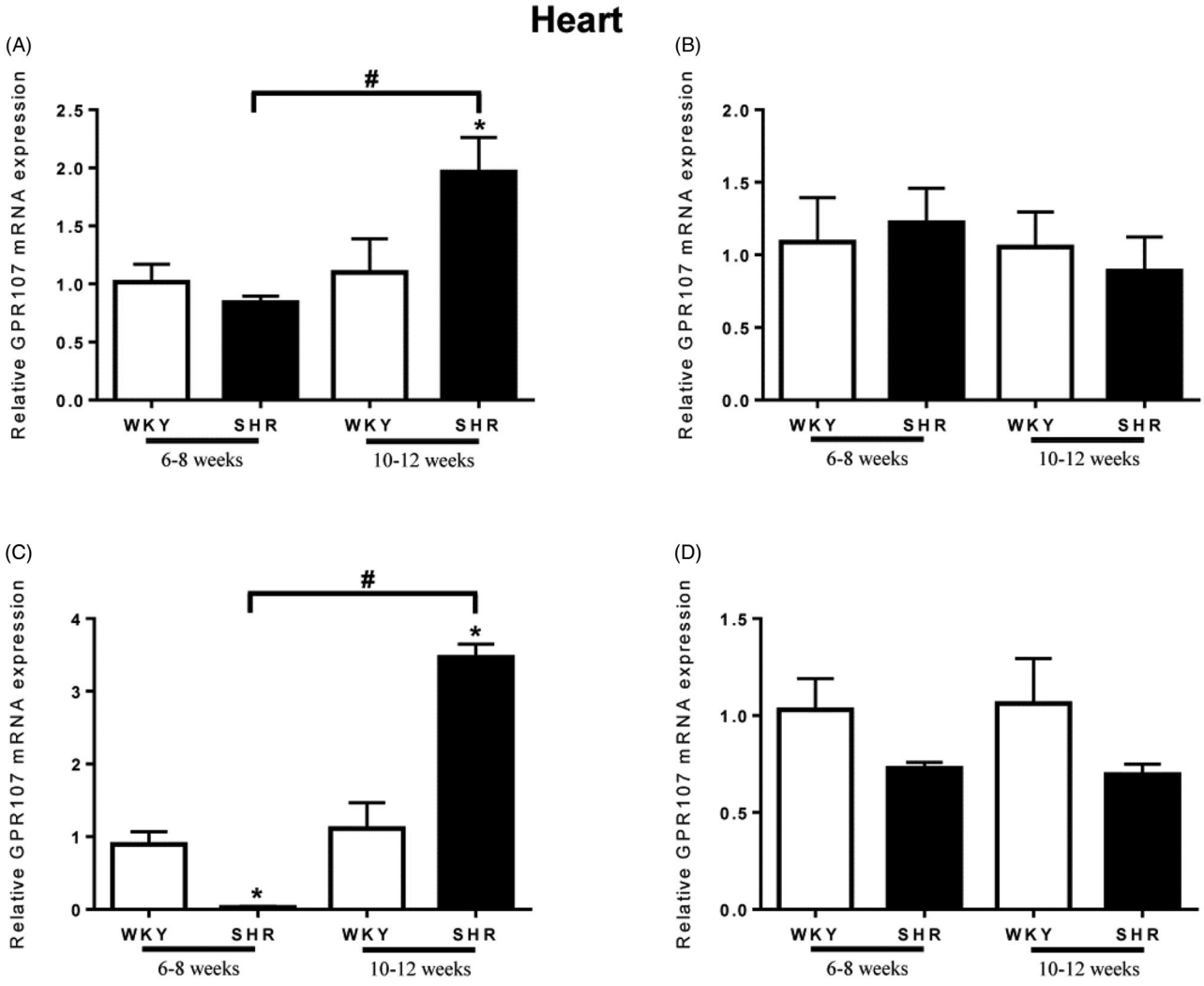

Figure 4. Relative expression of the orphan receptor GPR107 in (A) right atrium, (B) left atrium, (C) right ventricle and (D) left ventricle of rats with 6–8 and 10–12 weeks of age. Values are expressed as mean ± standard error. $^{*}p \leq .05$ vs WKY, $\#p \leq .05$ vs rats 6–8 weeks of each strain.

with hypertrophy and cardiomyopathy [43,44], which are cardiovascular consequences observed in spontaneously hypertensive rats due to changes in the expression of a series of genes in heart during the establishment of hypertension [45]. Consequently, we assume that GPR99 participates at the cardiovascular level in the establishment of hypertension in the SHR rat. In 2012 Yosten and coworkers [19] reported the expression of orphan receptor GPR107 in different rat tissues including kidney, heart and brain.

At the same time, we identified in our study the expression of GPR107 in the kidney, heart, brain and also aorta of SHR and WKY rats both young and adult, that is, we identified the expression of GPR107 during the development and establishment of hypertension in spontaneously hypertensive rats. Another important data reported by these same authors was the gene silencing against GPR107, because completely blocked the hypertensive effect that was generated in rats when a peptide called neuronostatin was administered; therefore, we suggest that this receptor could be regulating blood pressure.

The GPR107 receptor was also expressed in aorta showing an increase in this tissue at 6–8 weeks old, based on the bioinformatics analysis this receptor is coupled to a Gi/o protein, which suggests that an increase in the expression of this receptor in aorta it could be participating in the decrease of cyclic AMP levels, and as a result of this leads vasoconstriction causing high blood pressure. Some studies report receptors coupled to Gi protein when are activated in vascular smooth muscle induce vasoconstriction [46]; although this expression is only observed in the pre-hypertensive state. Finally, at heart, the expression levels of GPR107 in the atrium and left ventricle were very similar between both strains during the development and establishment of the disease; however, the expression of this receptor in the atrium and right ventricle was higher in hypertensive SHR rats. That is to say that an increase of receptor GPR107 coupled to Gi/o protein in the heart induces inhibition of cAMP and as a consequence a relaxation of cardiac muscle (negative chronotropic and inotropic effects). Cicala and coworkers [47] evaluated the effect on right ventricle in hypertensive patients and reported these patients to have a longer right ventricle relaxation time. These results coincide with some investigations that report an increase in heart Gi levels of 12 week SHR rats due to hypertension [48], such is the case of the GPR107 receptor. Meanwhile, SHR rats of 6–8 weeks they showed a decrease in GPR107 expression only in the

right ventricle, this may be due to the fact that during the development of hypertension in the SHR this receptor is exerting a cardioprotective effect similar to that of the β-adrenergic receptors coupled to Gi when their expression is low [49]. Therefore, we consider that the orphan receptor GPR107 participates at the vascular level in the development of hypertension and the cardiovascular level in the establishment of hypertension in the SHR rat.

Finally, we conclude that orphan receptors GPR99 and GPR107 are expressed in aorta, heart, kidney and brain in normotension and hypertension. However, the mRNA expression profile of these orphan receptors suggests an important role in cardiovascular functions, so we do not rule out that they could be directly or indirectly involved in the hypertension process, although it is necessary to carry out complementary studies to demonstrate clearly their participation in this pathology.

Disclosure statement

The authors report no declarations of interest.

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