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Authors: Stancho Stanchev, Lyubomir Gaydarski, Iva N. Dimitrova, Georgi Kotov, Boycho Landzhov, Vidin Kirkov, Alexandar Iliev

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Renal structural changes and apelin receptor expression in spontaneously hypertensive rats: implications for hypertension-induced kidney injury

Stancho Stanchev[1], Lyubomir Gaydarski[1], Iva N. Dimitrova[2], Georgi Kotov[3], Boycho Landzhov[1], Vidin Kirkov[4], Alexandar Iliev[1]

[1]Department of Anatomy, Histology and Embryology, Medical University of Sofia, Bulgaria
[2]Department of Cardiology, University Hospital 'St. Ekaterina', Medical University of Sofia, Bulgaria
[3]Clinic of Rheumatology, University Hospital 'St. Ivan Rilski', Department of Rheumatology, Medical Faculty, Medical University of Sofia, Bulgaria
[4]Department of Health Policy and Management, Faculty of Public Health 'Prof. Dr. Tzekomir Vodenicharov', Medical University of Sofia, Bulgaria

Address for correspondence: Dr. Stancho Stanchev, MD, PhD, Department of Anatomy, Histology and Embryology, Medical University of Sofia, Bulgaria, 2 'Zdrave' St., 1431 Sofia, Bulgaria; tel. +359 2 9172 609, e-mail: stanchev_1989@abv.bg

ABSTRACT

Background: Arterial hypertension is a primary risk factor for kidney disease. Recent advances have implied a potential link between the apelin system and renal homeostasis.

Materials and methods: We used 6- and 12-month-old spontaneously hypertensive rats and age-matched normotensive controls to assess the changes in the renal expression of the apelin receptor by immunohistochemical method. The study also evaluated correlations between the renal apelin receptor's expression and renal injury indicators.

Results: The histological analysis showed elevated glomerular sclerosis and tubulointerstitial damage indices in both groups of hypertensive rats compared to age-matched controls. Older rats within each group exhibited higher scores than younger ones. The immunohistochemical analysis revealed varying apelin receptor expression patterns, with tubular expression intensifyi

ng both with hypertension severity and age. Glomerular expression was notably higher in older hypertensive rats compared to normotensive controls. We reported significant positive c orrelations between glomerular apelin receptor expression and glomerular sclerosis index in older hypertensive animals. Similarly, a positive correlation between tubular apelin receptor e xpression and tubulointerstitial damage index was discovered in hypertensive rats, suggesting hypertension-related changes in apelin receptor expression and renal damage.
Conclusions: Our study found kidney changes and varying apelin receptor correlations in hyp ertensive rat kidneys, suggesting complex roles needing research.

Keywords: apelin receptor, hypertension, renal damage, chronic kidney disease, adaptive mechanism

INTRODUCTION

Arterial hypertension (AH) is a chronic medical condition and a primary risk factor for a number of pathologies, including cardiovascular diseases, cerebrovascular injury, and chronic kidney disease (CKD). It is among aging societies' foremost healthcare and financial burdens [21]. Although the precise etiology of hypertension remains to be completely understood, its development is associated with various factors [9, 40]. Hypertension-induced renal damage is a complex syndrome that significantly contributes to the development of CKD. The gradual deterioration of functional nephrons and alterations in the tubulointerstitium result in a decline in renal function [23, 31]. Evaluation of hypertension-induced renal injury can be effectively conducted utilizing markers such as the glomerular sclerosis index (GSI), tubulointerstitial damage index (TDI), and renal collagen content [2, 37, 38, 39, 43]. These parameters comprise various morphological alterations affecting the renal parenchyma and interstitium, including notable glomerulosclerosis characterized by an elevated proportion of shrinking and obsolete glomerular capillary tufts, arteriolar sclerosis, and hyalinosis. Furthermore, tubular atrophy accompanied by lumen dilatation, epithelium flattening, increased extracellular matrix expansion, and infiltration of inflammatory cells are evident [2, 19].

A natural peptide called apelin acts as a signaling molecule for the apelin receptor (APLNR), which belongs to the rhodopsin-like G-protein-coupled receptor family. Discovered in 1993, APLNR was initially considered an orphan until apelin was identified in 1998 [26]. Various apelin isoforms exist. Despite differences in length, all isoforms share the same 12 C-terminal

amino acid segments. Apelin is initially produced as a pre-pro-peptide with 77 amino acids before undergoing subsequent maturation [7].

In the kidneys of rats, the mRNA expression of APLNR has been previously studied [11, 24, 25]. The distribution pattern of APLNR shows increased expression in the medulla and minimal expression in the cortex [14]. O'Carroll et al. [24, 25] observed labeling corresponding to APLNR mRNA expression in 40% of the glomeruli within the kidney cortex, suggesting a potential regulatory role in blood flow. The role of the apelin/APLNR system is not entirely understood yet, although recent advances indicate that the apelin/APLNR system is of crucial importance in preventing renal aging [27]. Studies focusing on the vasculature, as conducted by Susztak et al., delineate the regulatory role of the apelinergic system in angiotensin II (Ang II) — AT1 receptor signaling [22, 34]. Plausibly, at the renal level, apelin may function as a counterregulatory element against the physiological actions of Ang II. Investigations into the role of apelin and APLNR in renal hemodynamics by Ishida et al. point to apelin's capacity to augment medullary blood flow through a vasodilatory mechanism, aligning with its antithetical effects to Ang II in terms of blood pressure [15, 30]. Moreover, apelin affects vascular tone, thus further improving medullary blood flow [10, 45].

Elabela (ELA) is a 54-amino-acid peptide which undergoes additional processing to generate mature peptides. The identification of ELA as the second endogenous ligand for APLNR elucidated the notable and unexpected differences in phenotypes observed between APLNR-knockout mice, characterized by non-Mendelian ratios at birth and significant cardiovascular developmental anomalies and apelin-knockout mice, which exhibited normal development [5].

Recent discoveries imply apelin's renoprotective effects, including its alleged capability to reduce interstitial fibrosis in the kidneys [37]. Furthermore, overexpression of ELA is reported to reduce renal fibrosis [28, 43]. However, the exact role of the apelinergic system in hypertension-induced kidney damage remains elusive. Therefore, the present study aims to investigate changes in the expression of APLNR in the kidneys of spontaneously hypertensive rats (SHR) as a model of hypertension-induced kidney injury. For that purpose, we assessed the immunohistochemical expression of APLNR in 6-month-old (corresponding to the initial hypertension stage) and 12-month-old SHR (advanced hypertension) and compared it with age-matched normotensive Wistar rats (WR) as controls. We also compared APLNR's expression within each of the two groups to assess its changes with the progression of hypertensive injury and physiological aging.

MATERIALS AND METHODS

Experimental animals

The study was conducted in the Department of Anatomy, Histology and Embryology at the Medical University of Sofia and approved by the Medical Legal Office and the Local Ethics Committee, based on ordeal No. 20/01.11.2012. Two age groups of SHR were implied in the current study: 6-month-olds corresponding to early or established AH and renal injury and 12-month-olds corresponding to advanced AH and renal injury [32]. Age-matched normotensive WR served as controls. Six male rats were randomly chosen from a larger pool of SHR and WR, respectively. Animal procedures followed previously established protocols [32]. Blood pressure, including systolic and diastolic measurements, was recorded using the tail-cuff method with a Model MK-2000ST device (Muromachi Kikai Co., Ltd., Tokyo, Japan) following the protocol of our previous studies [17].

Tissue preparation

The rats underwent intraperitoneal anesthesia with Thiopental at a dosage of 40 mg/kg b.w. Subsequently, the chest cavity was opened, and transcardial perfusion was performed using 4% paraformaldehyde. Following rapid extraction, the kidneys were fixed in a 10% neutral phosphate-buffered formalin solution for a minimum of 24 hours. The kidneys were sectioned parallel to their long axis, following standardized protocols [35]. Subsequently, kidney samples underwent dehydration using increasing concentrations of alcohol, clearing in xylene, and embedding in paraffin.

Evaluation of GSI and TDI

The renal histological changes were scored on PAS-stained sections for GSI and Mallory's trichrome stained sections for TDI. The evaluation of GSI and TDI was conducted on light microscopic examination of 20 glomeruli and 10 tubular fields per section, following established protocols outlined in previous studies [2, 32, 43]. Five sections were sampled from each kidney for analysis. Histological assessments were carried out independently by two investigators who were blinded to the slide origins, and mean values were computed for accuracy.

Immunohistochemistry

We performed an immunohistochemical study using the heat-induced epitope retrieval (HIER) technique as outlined in the methodology by Stanchev et al. [32]. We employed a mouse monoclonal anti-APLNR IgG antibody (Santa Cruz Biotechnology Catalogue No. sc-517300,

Santa Cruz Biotechnology, Inc., Heidelberg, Germany) at a concentration of 1:250. All subsequent steps and procedures adhered to the standardized protocol described in our previous study [32].

Semi-quantitative analysis

For the semi-quantitative assessment of APLNR expression, we employed ImageJ 1.52a software obtained from the National Institute of Health (NIH) website (http://imagej.nih.gov/ij/). Staining intensity was evaluated using the IHC Profiler plugin, downloaded from the Sourceforge website (https://sourceforge.net/projects/ihcprofiler/), following a well-established protocol [36]. The IHC Profiler categorized staining intensity into four tiers: high positive (3+), positive (2+), low positive (1+), and negative (0). We analyzed a minimum of ten random visual fields from each ventricle on every slide. Five slides were assessed from the heart of each animal within each age group. The final score for each ventricle within a specific age group represented the average score of all visual fields determined by the IHC Profiler.

Statistical analysis

The quantitative data collected were subjected to a statistical analysis starting with the one-way analysis of variance (ANOVA) and, consequently, post hoc analysis with the Tukey HSD test to ascertain the presence of significant differences in APLNR expression in the glomeruli and tubules of SHR compared to WR, as well as between 6-month-old and 12-month-old SHR, and between 6-month-old and 12-month-old WR. Additionally, the values of GSI and TDI of 6-month-old and 12-month-old SHR were compared to age-matched WR. Furthermore, APLNR expression in the glomeruli was juxtaposed with GSI in both age groups of SHR and WR, and the same comparison was made for APLNR expression in tubules with TDI. Pearson's correlation analysis was conducted to investigate potential correlations between APLNR expression and GSI/TDI. A standard level of significance of α (p-value) < 0.05 was utilized for all statistical tests.

Limitations

The current study had several limitations that merit acknowledgment. Firstly, APLNR expression in the kidney was only assessed semi-quantitatively. Additionally, it is essential to recognize the inherent variability in the visual quantification of immunohistochemistry slides among different observers. To address this concern, automated software was employed to

mitigate inter-observer inconsistencies, as demonstrated in prior studies [36]. Secondly, our investigation only included male SHR to mitigate potential confounding effects related to female sex hormones and periodic fluctuations observed in female SHR. Thirdly, it is noteworthy that the rat kidney exhibits a species-specific characteristic of being unipapillar, comprising only one pyramid, which may limit the generalizability of our findings to the human population. Lastly, histological evaluations, including assessing glomerular sclerosis and tubulointerstitial damage, are susceptible to variability and subjective interpretation, further contributing to the study's limitations.

RESULTS

Blood pressure measurement

Mean systolic and diastolic blood pressure values measured in SHR and WR are presented in Table 1.

Renal structural changes

The renal morphological alterations in SHR and WR were assessed with PAS reaction and Mallory's trichrome staining (Fig. 1 and 2).

GSI younger SHR was significantly higher compared to WR of the same age; the same result was reported in 12-month-old SHR compared to age-matched controls. When comparing younger and older animals in the two separate groups, 12-month-old rats in both the SHR and WR groups displayed a significantly higher GSI. Similar findings were reported for TDI as both 6- and 12-month-old SHR had significantly higher TDI compared to their normotensive counterparts. In the two separate groups, 12-month-old animals had a significantly higher TDI compared to 6-month-old ones.

The immunohistochemical reactivity of APLNR in the kidney was semi-quantitatively assessed (Tab. 2). In 6-month-old SHR, most slides showed mainly negative APLNR expression in the glomeruli, with low positive expression predominating in the parietal layer of Bowman's capsule (Fig. 3A). No significant difference in the distribution was reported in 6-month-old WR, where most slides showed negative APLNR expression in the glomeruli (Fig. 3B). In 6-month-old SHR, APLNR expression in the tubules was negative, with a marginally less than half of slides showing low positive expression (Fig. 3C), which was slightly higher than the predominantly negative expression in the control group (Fig 3D) but not statistically significant. In 12-month-old SHR, most slides showed positive and a few — high positive expression of APLNR in the glomeruli, predominantly in the endothelium of

glomerular capillary tufts, on the visceral layer and to a lesser extent on the parietal layer of Bowman's capsule (Fig. 4A). These findings differed significantly from glomerular APLNR expression in 12-month-old WR controls (Fig. 4B). APLNR tubular expression was predominantly observed in the membrane of epithelial cells lining the proximal and distal tubules in the renal cortex. In the renal medulla, APLNR expression appeared again on the membrane of the epithelial cells lining the proximal and distal part of the loop of Henle, as well as the epithelium of the collecting ducts. Mainly positive expression was noted in the tubules of 12-month-old SHR (Fig. 4C), which appeared higher than the control group (Fig. 4D) but not statistically significant. APLNR expression in tubules significantly increased with deterioration of AH and age progression. In the glomeruli, a significant difference in APLNR expression was found between 6- and 12-month-old SHR, while no significant difference was observed between 6- and 12-month-old WR. Moreover, when comparing the expression of GSI/APLNR between different groups, notably between 6-month-old SHR and 12-month-old SHR, a highly significant difference was observed. Similar patterns of significance were also observed in the comparison of TDI/APLNR expression between different groups. The results of the post hoc Tukey HSD test are summarized in Tables 3 and 4.

Correlation analysis

The results of the correlation analysis are summarized in Table 5. When juxtaposing GSI and APLNR expression in 6-month-old SHR, a weak to moderate positive correlation was found, which was not statistically significant. Similarly, in 6-month-old WR, a weak positive correlation was observed, again not statistically significant. In 12-month-old SHR, a moderate positive correlation was observed, which was statistically significant. However, in 12-month-old WR, a weak positive correlation was observed, but it was not statistically significant.

Furthermore, correlations were examined between APLNR expression in tubules and TDI. In 6-month-old SHR, a weak to moderate positive correlation was observed, but it was not statistically significant. In 6-month-old WR, a weak positive correlation was observed, again not statistically significant. In contrast, in 12-month-old SHR, a moderate positive correlation was observed, which was statistically significant. Likewise, in 12-month-old WR, a moderate positive correlation was observed, which was statistically significant.

DISCUSSION

Our findings shed light into the immunochemical localization of APLNR in the kidney. APLNR's immunoreactivity was mainly visualized on the membrane of epithelial cells lining

the tubules in both age groups of SHR and WR, which is in line with the results of Sekerci et al. [29]. An intriguing discovery was the presence of APLNR in the capillary tufts, visceral, and parietal layer of Bauman's capsule. Although APLNR's immunoreactivity was visualized in the glomeruli of all experimental animals, the most profound expression was registered in the glomeruli of 12-month-old SHR. This finding is in direct contrast with the results of Sekerci et al. [29], who reported the absence of APLNR expression in the glomeruli of both hypertensive and normotensive rats. Moreover, we evaluated two parameters of hypertension-induced renal damage — GSI and TDI. Both indices were higher in SHR compared to age-matched WR. Statistically significant results were observed in several comparisons. Firstly, there was a significant increase in APLNR expression within the glomeruli of 12-month-old SHR compared to normotensive controls. Additionally, both GSI and TDI were significantly higher in both young and old SHR compared to age-matched controls. Moreover, both indexes were significantly higher in 12-month-old SHR compared to 6-month-old SHR. The same trend was observed in old WR compared to younger WR, although not as pronounced as in the SHR group. All comparisons of both GSI and TDI were found to be statistically significant. However, it is worth noting that while APLNR expression was higher in the tubules of both groups of SHR compared to WR controls, this difference was not statistically significant. Similarly, while there was a marginally higher expression of APLNR in the tubules of 12-month-old SHR compared to those of 6-month-old SHR, this difference was not statistically significant either. Furthermore, the correlation analysis unveiled varying degrees of correlation between APLNR expression in glomeruli and GSI across SHR and WR of both age groups. However, only 12-month-old SHR showcased a moderate, statistically significant positive correlation with a $r^2 = 0.1531$, which explains 15% of GSI rise during AH progression with the upregulation of APLNR's expression. Similarly, the results of the correlation analysis reviewed differing levels of correlation between APLNR's expression in tubules and TDI in both age groups of SHR and WR. 12-month-old SHR exhibited a moderate positive correlation with $r^2 = 0.1344$, which was statistically significant and explained 13% of the increased TDI with the upregulation in the expression of APLNR with deterioration of AH. Likewise, 12-month-old WR demonstrated a moderate positive statistically significant correlation with $r^2 = 0.1045$, explaining 10% of the TDI with the upregulation of APLNR in tubules. Our study's unique aspect involves examining how APLNR expression in glomeruli and tubules changes with the advancement of hypertensive-induced renal injury and aging and correlating these changes with GSI and TDI values, which are morphometric indexes for renal

injury. This investigation sheds light on the role of APLNR in the complex compensatory mechanisms involved in the progression from AH to CKD.

In order to better comprehend the merit of our findings, we need to review the role of the apelinergic system in the kidney. In rats, apelin has been shown to counteract the vasoconstriction triggered by Ang II in both the afferent and efferent arterioles, rapidly reducing intracellular calcium levels. This vasodilatory response to apelin is contingent upon an intact endothelium and nitric oxide production [14]. Considering that the vasa recta receive their blood supply from the efferent arteriole, the vasodilatory effect induced by apelin can potentially augment diuresis by enhancing renal medullary blood flow [4]. Furthermore, apelin directly impacts the renal tubule, stimulating diuresis in a dose-dependent manner without affecting the handling of renal sodium or potassium [1, 14]. Both in vitro and in vivo investigations suggest that this effect is mediated through inhibiting the aquaporin 2 channel [1, 13]. Structural analyses have revealed specific interactions between residues of pE13F and acidic amino acids on the surface of APLNR [8, 16]. Several studies have investigated the signaling pathways these apelin variants activate, demonstrating their ability to inhibit cyclic adenosine monophosphate (cAMP) production and increase calcium mobilization across diverse cell types [8, 16]. Moreover, apelin peptides induce vasodilation and regulate vascular tone by producing nitric oxide [8]. Activation of the apelin/APLNR system initiates intracellular signaling cascades involving the PI3K/Akt and ERK1/2 pathways, leading to the phosphorylation of S6 ribosomal protein kinase (p70S6K). The internalization of APLNR occurs through a clathrin-dependent mechanism, mediated by interactions with β-arrestins and specific phosphorylation events [3, 16]. Functional investigations suggest that different apelin peptides can activate distinct signaling pathways and elicit varied biological responses, indicating functional selectivity or biased signaling of APLNR. Additionally, the trafficking and desensitization of APLNR are influenced by the ligand that activates it [3]. APLNR can also form heterodimers with other G protein-coupled receptors (GPCRs), influencing various cellular processes such as cell proliferation and signaling [33]. The mRNA of APLNR has been identified in both endothelial cells and vascular smooth muscle cells within rat glomerular arterioles [14]. The expression of APLNR mRNA is notably high in glomeruli, nearly eight times greater than in tubules [14]. The highest levels of APLNR mRNA were detected in the medulla [24, 25, 29, 41, 42].

It seems the rodent kidney has more prominent expression of ELA than apelin exhibits a higher expression in the rodent kidney than apelin, with a predominant localization in the medullary collecting ducts [24, 25]. Despite this abundance, the precise mechanism through

which ELA induces diuresis remains unclear. However, the constrained expression of ELA to the vascular endothelium in the kidney implies renoprotective effect [4]. In laboratory studies, it has been demonstrated that the apelin system modulates the expression of the intrarenal renin-angiotensin system (RAS). For instance, in cells derived from the collecting duct, ELA-32 reduced the expression of renin and its precursors [42]. Animal models further support the reciprocal regulation between RAS and apelin/APLNR systems. For example, in Dahl salt-sensitive rats, the infusion of ELA-32 prevented the upregulation of renin, AT1 receptor, and AT2 receptor mRNA induced by a high-salt diet [42]. This might serve as a plausible explanation for the observed changes in the expression of APLNR with the progression of AH. Even though the current study only focuses on APLNR's expression, one potential hypothesis might be that it becomes elevated as a desperate attempt to compensate for the depleting levels of the endogenous ligands (apelin and ELA forms). Overall, there is currently no direct evidence supporting the notion that the expression of APLNR in kidneys responds to changes in plasma apelin levels. Nevertheless, investigations indicate that the distribution of apelin and APLNR within the kidney is heterogeneous and subject to modulation by various factors, including disease conditions and experimental variables [4, 24]. Xu et al. [42] observed an upregulation of APLNR in SHR aorta and smooth muscle cells alongside a concomitant downregulation of apelin. Conversely, Sekerci et al. [29] reported decreased apelin levels and APLNR downregulation in the kidney. Hence, further investigation is warranted to elucidate if there is an association between the expression of APLNR and the plasma levels of its ligands.

Renal interstitial fibrosis is a complex process influenced by various factors that disrupt the balance between collagen synthesis and degradation [44]. The deterioration of renal damage in SHR is pressure-dependent and associated with disturbed sympathetic activity [12, 39]. Research by Chatziantoniou et al. has highlighted several factors implicated in renal fibrosis in the context of AH [6]. Additionally, oxidative stress has been identified by Zhao et al. [44] as a contributor to the profibrogenic phenotype in hypertension-induced CKD. These findings underscore the critical role of renal fibrosis in the pathogenesis of AH-associated CKD.

The progression of CKD often culminates in glomerulosclerosis and interstitial fibrosis, representing a common final outcome. In the early phases of renal damage, there's an increase in inflammatory and pro-fibrotic agents, leading to advancing fibrosis and a subsequent decrease in glomerular filtration rate (GFR). RAS notably participates in renal fibrosis through Ang II's interaction with the AT1 receptor. Inhibiting this system has been shown to be beneficial in the management of CKD [18, 23, 39]. Apelin demonstrates antifibrotic effects

[37], and the overexpression of ELA or administration of the ELA-32 peptide reduces renal fibrosis and inflammation in salt-sensitive rats [28, 42]. In a model of kidney fibrosis induced by unilateral ureteric obstruction, the apelin system shows upregulation in the damaged kidney.

All this suggests a central role of the apelinergic system in the antifibrotic processes, thus implying a strong renoprotective function. In the present study, however, correlations between APLNR expression in the kidney and two widely used markers of renal fibrosis and damage, GSI and TDI, were not statistically significant during the onset of AH (6-month-old SHR), nor in the age-matched controls (6-month-old WR). Moreover, it is worth noting that a positive, statistically significant correlation between APLNR's expression in the glomeruli and GSI was recorded in 12-month-old SHR (established AH). According to our analysis, 15% of the GSI increase could be explained by the elevation in APLNR's expression in old SHR, compared to only 6% of the GSI rise that could be attributed to APLNR's expression in the age-matched WR controls. Therefore, even though our study demonstrated a significant upregulation of APLNR in the glomeruli and non-significant elevation of APLNR in the tubules in the context of hypertension-induced kidney injury, the conducted correlation analysis implies that APLNR alone may not be the primary factor involved in either the progression of structural injury or compensatory mechanisms activated to counter it. Nevertheless, our study provides valuable insight into the delicate interplay of the apelinergic system and, more specifically, APLNR in the regulatory and compensatory mechanisms taking place during AH development and deterioration. These findings pave the way for future research on the role of the apelinergic system in the sophisticated vascular adaptive mechanisms occurring in the hypertensive kidney.

CONCLUSIONS

In summary, our study revealed significant structural changes in the kidneys of SHR, accompanied by elevated expression of APLNR, particularly in the glomeruli of the advanced stage of hypertension-induced kidney injury. However, correlations between APLNR's expression and renal injury indicators were to a different extent in the two age groups of WR and SHR and could only explain up to 15% of the structural changes with the upregulation of APLNR's expression. These findings suggest a more complex role for the apelin system in hypertension-induced renal damage, warranting further research to elucidate its precise mechanisms and therapeutic implications for managing this condition.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon request.

Ethics statement

This study was approved by the Medical-Legal Office and the Local Ethics Committee.

Author contributions

S.S.—project development, data collection and management, data analysis, and manuscript writing. L. G.—data collection and analysis and manuscript writing. I.N.D.—data analysis and manuscript editing. G.K.—data analysis and manuscript editing. B.L.—data analysis and manuscript editing. V.K.—data analysis and manuscript editing. A.I.—data analysis, data collection, and manuscript writing. All authors have met the criteria for authorship as established by the International Committee of Medical Journals Editors, believe that the paper represents honest work, and can verify the validity of the results reported. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflicts of interest.

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Table 1. Mean systolic (mmHg) and mean diastolic blood pressure (mmHg) of 6- and 12-month-old spontaneously hypertensive rats.

Age group	**Mean systolic blood pressure (mmHg) ± SD**	**Mean diastolic blood pressure (mmHg) ± SD**
6-month-old WR	110 ± 2.6	78.2 ± 2.4
12-month-old WR	121 ± 3.5	82.9 ± 3.6
6-month-old SHR	176.6 ± 2.9	112.1 ± 3.1
12-month-old SHR	199.3 ± 3.2	126.5 ± 2.2

Each group consisted of six animals (n = 6). SD — standard deviation; SHR — spontaneously hypertensive rats; WR — Wistar rats.

Table 2. Semi-quantitative analysis of the intensity of immunohistochemical staining for the apelin receptor in the renal cortex and renal medulla in 6- and 12-month-old Wistar rats and spontaneously hypertensive rats.

APLNR		**SHR**	**WR**
6-month-old rats	Renal corpuscles	High-positive (3+) (0%) Positive (2+) (0%) Low-positive (1+) (28%) Negative (0) (72%)	High-positive (3+) (0%) Positive (2+) (0%) Low-positive (1+) (21%) Negative (0) (79%)
	Renal tubules	High-positive (3+) (0%) Positive (2+) (0%) Low-positive (1+) (44%) Negative (0) (56%)	High-positive (3+) (0%) Positive (2+) (0%) Low-positive (1+) (23%) Negative (0) (76%)
12-month-old rats	Renal Corpuscles	High-positive (3+) (8%) Positive (2+) (58%) Low-positive (1+) (20%) Negative (0) (14%)	High-positive (3+) (0%) Positive (2+) (0%) Low-positive (1+) (28%) Negative (0) (72%)

	Renal tubules	High-positive (3+) (9%) Positive (2+) (66%) Low-positive (1+) (12%) Negative (0) (13%)	High-positive (3+) (0%) Positive (2+) (72%) Low-positive (1+) (17%) Negative (0) (11%)

The analysis was performed by immunohistochemistry profiler. APLNR — apelin receptor; SHR — spontaneously hypertensive rats; WR — Wistar rats.

Table 3. Descriptive statistics of the ratio between 6- and 12-month-normotensive Wistar rats (WR) and 6- and 12-month-old spontaneously hypertensive rats (SHR) for GSI, TDI and APLNR expression.

Compared groups	**GSI/GSI**	**TDI/TDI**	**APLNR-C/APLNR-C**	**APLNR-T/APLNR-T**
6mWR/6mSHR	p = 0.000019	p = 0.03	p = 0.9	p = 0.9
12mWR/12mSHR	p < 0.000001	p = 0.0002	p = 0.000082	p = 0.9
6mWR/12mWR	p < 0.000001	p = 0.06	p = 0.543	p = 0.1
6mSHR/12mSHR	p < 0.000001	p = 0.0006	p = 0.000001	p = 0.2

APLNR-C — expression of apelin receptor in corpuscles; APLNR-T — expression of apelin receptor in tubulointrestitium; GSI — glomerular sclerosis index; p — p-value; TDI — tubulointerstitial damage index.

Table 4. Descriptive statistics of the ratio between GSI, TDI and APLNR expression in 6- and 12-month-normotensive Wistar rats (WR) and 6- and 12-month-old spontaneously hypertensive rats (SHR) for.

Compared groups	**6mWR**	**6mSHR**	**12mWR**	**12mSHR**
GSI/APLNR-C	P = 0.7	P = 0.000004	P < 0.000004	P < 0.000001

TDI/APLNR-T	P = 0.9	P = 0.06	P = 0.9	P = 0.00005

APLNR-C — expression of apelin receptor in corpuscles; APLNR-T — expression of apelin receptor in tubulointrestitium; GSI — glomerular sclerosis index; p — p-value; TDI — tubulointerstitial damage index.

Table 5. Descriptive correlation analysis of the ratio between 6- and 12-month-normotensive Wistar rats (WR) and 6- and 12-month-old spontaneously hypertensive rats (SHR) for GSI, T DI and semi-quantitative assessment of APLNR expression.

Correlated parameters	**r**	**r^2**	**p-value**
APLNR-C 6m SHR/GSI 6m SHR	0.2697	0.07273	0.1495
APLNR-C 6m WR/GSI 6m WR	0.1712	0.0293	0.3657
APLNR-C 12m SHR/GSI 12m SHR	0.3876	0.1531	0.03566
APLNR-C 12m WR/GSI 12m WR	0.2357	0.05556	0.2099
APLNR-T 6m SHR/TDI 6m SHR	0.2896	0.08385	0.1206
APLNR-T 6m WR/ TDI 6m WR	0.2344	0.05495	0.2125
APLNR-T 12m SHR/TDI 12m SHR	0.3734	0.1344	0.04212
APLNR-T 12m WR/TDI 12m WR	0.3554	0.1045	0.04547

APLNR-C — expression of apelin receptor in corpuscles; APLNR-T — expression of apelin receptor in tubulointrestitium; GSI — glomerular sclerosis index; r — Pearson correlation coe fficient; r^2 — coefficient of determination; TDI — tubulointerstitial damage index.

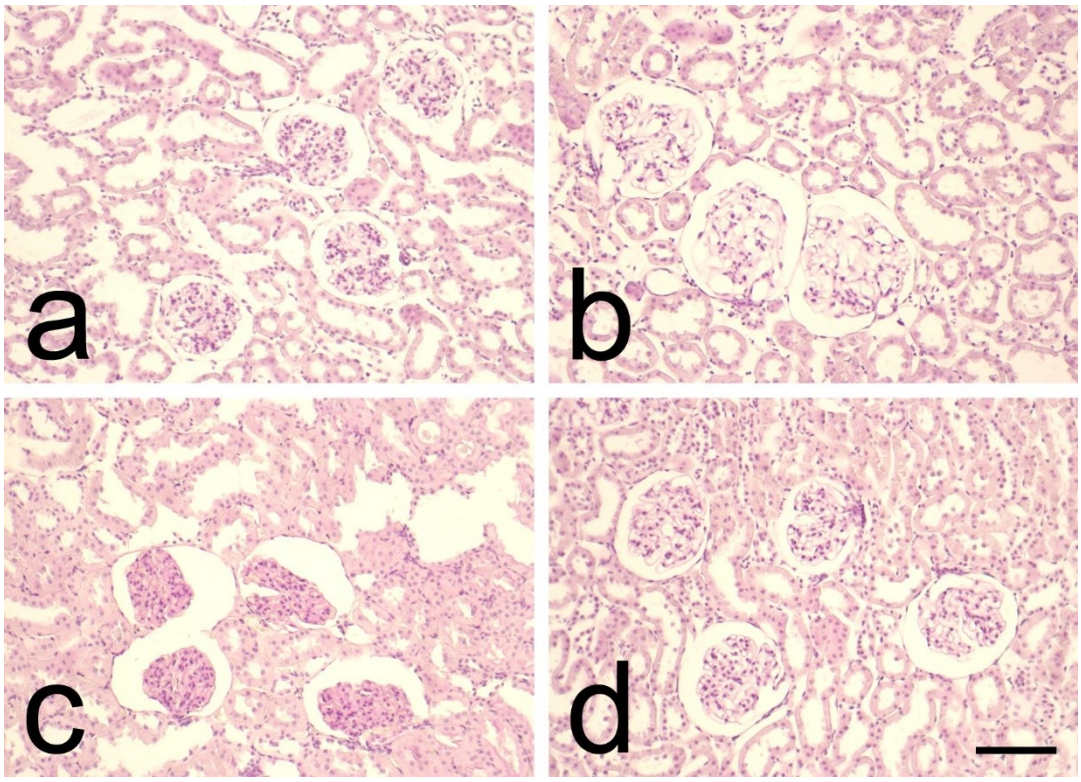

Figure 1. Renal morphological changes in the kidneys of 6- and 12-month-old spontaneously hypertensive rats (SHR) and Wistar rats (WR) observed on sections stained with periodic acid-Schiff (PAS). **A.** 6- month-old SHR; **B.** 6-month-old WR; **C.** 12-month-old SHR; **D.** 12-month-old WR.; scale bar 50 μm.

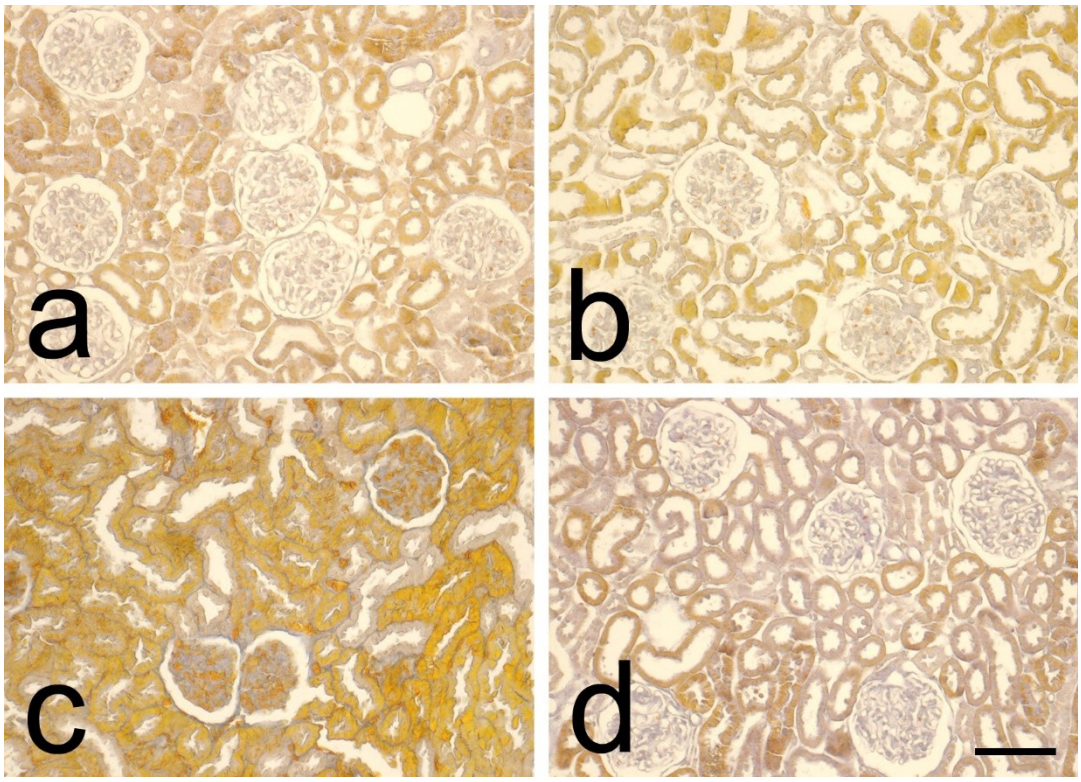

Figure 2. Renal morphological changes in the kidneys of 6- and 12-month-old spontaneously hypertensive rats (SHR) and Wistar rats (WR) observed on sections stained with Mallory's trichrome stain. **A.** 6- month-old SHR; **B.** 6-month-old WR; **C.** 12-monthold SHR; **D.** 12-month-old WR; scale bar 50 μm.

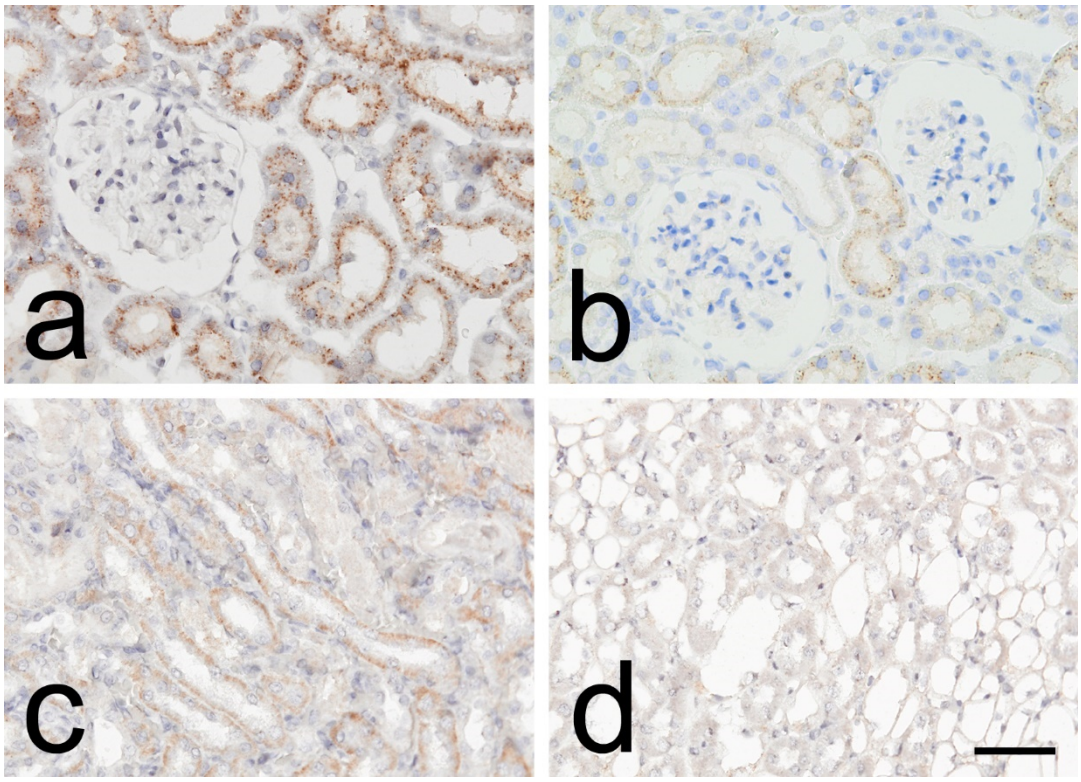

Figure 3. Immunohistochemical staining for apelin receptor (APLNR) in the kidney of 6-month-old spontaneously hypertensive rats (SHR) and 6-month-old normotensive Wistar rats (WR). **A.** Renal cortex (RC) of 6-month-old SHR; **B.** Renal cortex (RC) of 6-month-old WR; **C.** Renal medulla (RM) of 6-month-old SHR; **D.** Renal medulla (RM) of 6-month-old WR; scale bar 25 μm.

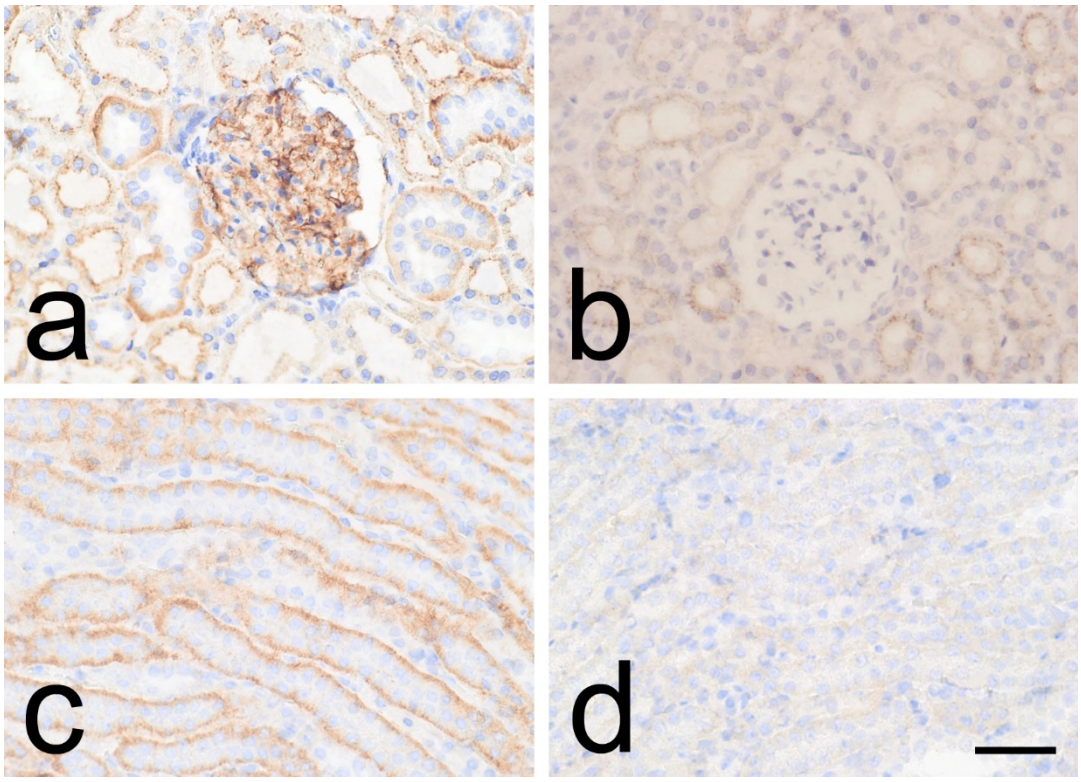

Figure 4. Immunohistochemical staining for apelin receptor (APLNR) in the kidney of 12-month-old spontaneously hypertensive rats (SHR) and 12-month-old normotensive Wistar rats (WR). **A.** Renal cortex (RC) of 12-month-old SHR; **B.** Renal cortex (RC) of 12-month-old WR; **C.** Renal medulla (RM) of 12-month-old SHR; **D.** Renal medulla (RM) of 12-month-old WR; scale bar 25 μm.