INTERACTION OF KIDNEY PROTEINS OF NORMAL AND HYPERTENSIVE RATS WITH FRAGMENTS OF RENALASE PEPTIDE RP220

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Renalase (RNLS) is a protein involved in the regulation of blood pressure; it has various functions inside and outside cells. The twenty-membered peptide RP220, corresponding to the amino acid sequence of human RNLS 220–239, reproduces a number of effects of extracellular RNLS and can bind to many intracellular proteins in the kidney. The RP220 sequence contains several cleavage sites for extracellular proteases, which could potentially produce RP224-232 and RP233-239 peptides. The aim of this work was to perform proteomic profiling of kidney tissue from normotensive Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) derived from WKY, using potential proteolytic fragments (RP224-232 and RP233-239) of the RP220 peptide as affinity ligands, and to compare these proteomic profiles with the profiles obtained using the parent RP220 peptide. The obtained results indicate that the relative content of proteins bound to the RNLS peptides in SHR, compared to that in WKY rats, changes most significantly in the case of the RP224-232 peptide. Almost all of these proteins, with a few exceptions, are associated with cardiovascular pathology, many with hypertension. The results of our work indicate that proteolytic processing of RP220 does not lead to the inactivation of this peptide, but to a change in its ligand/regulatory properties, as well as the repertoire of potential protein partners and, consequently, protein-protein interactions that may have possible pharmacological application.

Keywords: renalase; renalase peptides RP220, RP224-232, and RP233-239; arterial hypertension; WKY and SHR rats; proteomic profiling of kidney tissue

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INTRODUCTION

Renalase (RNLS) is a secretory protein discovered twenty years ago; it is involved in the regulation of blood pressure, exerting its regulatory effects through catalytic and non-catalytic mechanisms [1–5]. Full-length intracellular RNLS is a FAD-dependent oxidoreductase (EC 1.6.3.5) that catalyzes the oxidation reaction of isomeric forms of β-NAD(P)H reduced at positions 2 or 6 of the nicotinamide moiety instead of the metabolically active position 4 [6]. Extracellular RNLS, lacking its N-terminal signal peptide, apparently undergoes proteolytic processing [7] to form RNLS peptides, which mediate the effects of this extracellular protein.

One of the most interesting RNLS peptides that attract much attention of researchers is RP220, a 20-membered peptide corresponding to the amino acid sequence of RNLS 220–239 [5, 8]. Its critical regulatory role is supported by the fact that this peptide reproduces a number of effects of extracellular RNLS [5, 8]. It should be noted that the RP220 sequence contains several cleavage sites for extracellular and circulating proteases, which can form several peptides, including RP224-232 and RP233-239, corresponding to fragments of the RNLS sequence 224–232 and 233–239, respectively [7, 9].

A biosensor study of the interaction of model intracellular proteins that bind to the RP220 peptide [10] showed that the RP224-232 peptide also interacted with the studied model proteins, demonstrating an affinity comparable to that of RP220. The latter indicates that the amino acid sequences, crucial for RP220 interaction with its (at least model) targets, included residues 224-VSIDNKKRN-232 [9].

This suggested that possible proteolytic cleavage of the twenty-membered RP220 peptide to form the RP224-232 peptide had little effect on the qualitative composition of the protein fractions bound to these peptides [9]. However, the interaction of RP233-239 with potential tissue targets was not studied. In this regard, the aim of the present work was a comparative proteomic profiling of kidney tissue from normotensive and hypertensive rats using peptides RP224-232 and RP233-239 as affinity ligands.

MATERIALS AND METHODS

Reagents

The following reagents were used in this study: cyanogen bromide-activated Sepharose 4B, ammonium bicarbonate, dithiothreitol, guanidine hydrochloride, urea, sodium chloride, Triton X-100, 4-vinylpyridine,

Coomassie Brilliant Blue G-250 (Merck, USA); formic acid, sodium acetate, boric acid, sodium tetraborate, sodium hydroxide (Acros Organics, USA), acetonitrile (Fisher Chemical, UK); isopropanol, trifluoroacetic acid (Fluka, USA); tris-(2-carboxyethyl)-phosphine (Pierce, USA); modified trypsin (mass spectrometry grade, Promega, USA). The nine-membered RNLS peptide RP224-232 (VSIDNKKRN) and the seven-membered RNLS peptide RP233-239 (IESSEIG), corresponding to the fragments of the human renalase peptide RP220-239 (CIRFVSIDNKKRNIESSEIG), were synthesized by BelkiAntitela (Russia). The purity of each of these peptides was 98%. Other reagents of the highest purity grade available were from local suppliers.

Experimental Animals

The experiments were performed on 14-week-old male normotensive Wistar Kyoto rats (WKY) (n=6; blood pressure 110–120 mmHg) and spontaneously hypertensive rats (SHR) (n=5; blood pressure 180+ mmHg) obtained from the Pushchino laboratory animal nursery (branch of the M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences). The animals were decapitated under light ether anesthesia; the kidneys were quickly removed, immediately frozen and stored at -70°C until the study.

Preparation of Lysates of Kidney Homogenates

Kidney tissue was homogenized using a Heidolph SilentCrusher homogenizer (50,000 rpm) in 0.05 M potassium phosphate buffer (pH 7.4) (buffer A) and diluted with this buffer to a final concentration of 30 mg/ml. To assess the relative quantitative changes in the protein content, the same amount of total protein was used during sample preparation; the total protein content was controlled using the Bradford method [11]. After incubation in the presence of 3% Triton X-100 (4°C, 1 h), the lysates were diluted 3-fold with the same buffer and centrifuged for 30 min at 16,000 *g* to obtain a cleared supernatant.

Affinity Chromatography of Rat Kidney Proteins Using RP220 Peptide Fragments Immobilized on Cyanogen Bromide-Activated Sepharose 4B

Covalent binding of the RP220 peptide fragments RP224-232 and RP233-239 to cyanogen bromide-activated Sepharose 4B (CNBr-Sepharose) was performed according to the standard protein/peptide ligand immobilization protocol [12]. To determine proteins that non-specifically bind to the affinity sorbent, we have used control cyanogen bromide-activated Sepharose, which was subjected to the same procedures, but without the addition of RNLS peptides.

Rat kidney tissue lysate at a concentration of 2 mg/ml was added to the affinity carrier washed with buffer A. Samples were incubated overnight in suspension (1:1) at 4°C with gentle mixing (with the addition of a protease inhibitor cocktail at the concentration recommended by the manufacturer). Subsequent procedures were carried out at room temperature. After washing of non-specifically bound proteins with buffer A, containing 0.3 M NaCl, until protein disappearance in the washing waters (control according to D_{280}), elution was carried out with 0.2 M glycine buffer, pH 2.8, containing 0.5 M NaCl (elution rate was 0.5 ml/min). The eluate was concentrated to a volume of 250 µl using Amicon Ultra membrane filters, as recommended by the manufacturer. Then the proteins were precipitated with a chloroform-ethanol mixture, as described in [13].

Identification and Comparison of the Relative Content of Proteins from WKY and SHR Rats Bound to Immobilized Fragments of the RP220 Peptide

Sample preparation for mass spectrometric analysis (reduction of disulfide bonds, alkylation of sulfhydryl groups, and trypsinolysis) was performed on membrane centrifuge filters as described in [14].

Mass spectrometric analysis was performed using the equipment of the Human Proteome Shared Use Center at the Institute of Biomedical Chemistry (IBMC). The conditions of mass spectrometric analysis are described in [15].

RESULTS AND DISCUSSION

Proteomic profiling of kidney proteins, bound to the fragments of the RP220 peptide, revealed a large group of SHR proteins demonstrating increased relative content compared to that of normotensive rats (WKY). For the RP224-232 peptide, it included 106 proteins, and for the RP233-239 peptide 118 proteins were identified (Table 1, Fig. 1, Tables S1 and S3 of the Supplementary Materials). In both cases, this number was somewhat higher than in the case of the RP220 peptide (98) [10]. In all three cases of the RNLS derived peptides as the affinity ligands, the proteins involved in the regulation of gene expression, cell division, and differentiation predominated among the SHR kidney proteins demonstrating increased relative content versus WKY. The number of RP220 binding proteins, which were characterized by decreased relative content in SHR rats, was significantly higher than in the case of its fragments RP224-232 and RP233-239 (90, 55, and 14, respectively) (Table 1, Fig. 1, Tables S2 and S4 of the Supplementary Materials). In the case of both the RP220 peptide and its RP224-232 fragment, proteins involved in the regulation of gene expression, cell division, and differentiation, as well as proteins of cytoskeleton, transport, and exocytosis, predominated (Table 1).

Table 1. Functional distribution of SHR kidney proteins bound to RP220-, RP224-232-, and RP233-239 peptides and demonstrating altered relative content compared to that of normotensive WKY rats*

Functions	Number of SHR proteins with increased relative content versus that of WKY			Number of SHR proteins with decreased relative content versus that of WKY		
	RP220	RP224-232	RP233-239	RP220	RP224-232	RP233-239
Energy generation and carbohydrate metabolism	14	16	23	8	6	1
Cytoskeleton, transport, and exocytosis	12	9	19	30	25	3
Signal transduction and enzyme activity regulation	10	8	10	10	4	2
Antioxidant and protective proteins	15	16	21	8	5	1
Regulation of gene expression, cell division, and differentiation	33	49	33	31	14	6
Metabolism of proteins, amino acids, and other nitrogenous substances	10	5	8	3	1	0
Lipid metabolism	4	3	4	0	0	1
Total	98	106	118	90	55	14

The table compiled using data of Supplementary Materials Tables S1–S4. Results obtained using RP220 were taken from [10].

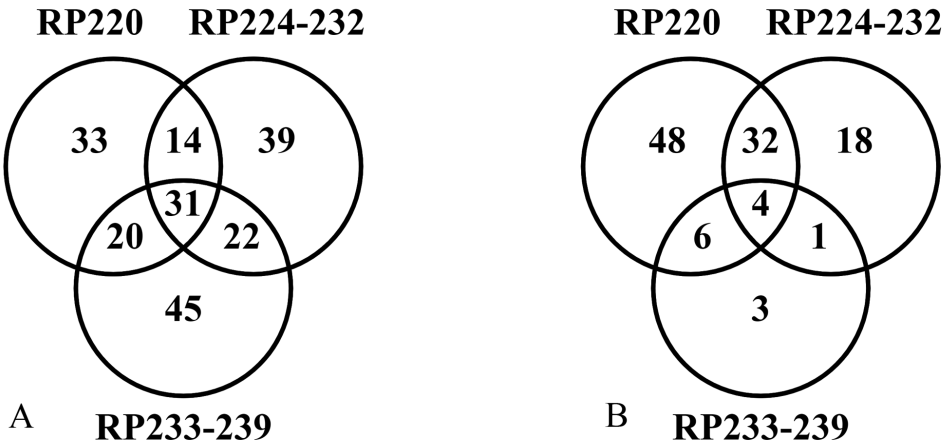

Figure 1. Kidney proteins bound to the RP220 peptide and its fragments RP224-232 and RP233-239 and demonstrating altered relative content in SHR compared to normotensive WKY rats: **A** – increased relative content. **B** – decreased relative content.

Proteins with higher relative abundance in SHR are evenly distributed among sets common to all three peptides RP220, RP224-232, and RP233-239 and individual sets for each of these peptides (31, 33, 39, and 45 proteins, respectively). Forty-five common proteins with altered relative abundance in hypertensive animals compared to normotensive (WKY) rats were identified for the RP220 peptide and its RP224-232 fragment; 51 common proteins were identified for the RP220 peptide and its RP233-239 fragment (Fig. 1A). The number of proteins with decreased relative content in SHR rats, common for the RP220 peptide and its RP224-232 fragment (36 proteins), was also comparable with the number of corresponding proteins sorbent-specific for the RP220 peptide (48), and exceeded the number of proteins specific for the RP224-232 peptide (18). In the case of the RP233-239 peptide, the number of proteins with decreased relative content in SHR, compared to WKY rats, was rather small (only 14) (Table 1, Fig. 1B).

The change in the relative content of SHR rats kidney proteins, compared to WKY rats, was especially demonstrative in the case of the RP224-232 fragment. For example, for fructose-1,6-bisphosphatase 1, the relative content increased by more than 2000 times. The relative content of the 60S subunit ribosomal proteins L4, L6, L7a, L8, L9, L11, L12, L18, L18a, L22, L23a, L30 demonstrated a significant increase (from 20 to more than 230 times); the similar trend was also found in the case of the 40S subunit ribosomal

proteins S3, S4, S7, S8, S12, S13, S15a, S20, S23, S25 (from 20 to almost 100 times) (Table 2, Fig. 2). The relative content of catalase, bound to the RP224-232 peptide, increased in hypertensive rats by more than 230 times, glutathione-S-transferase alpha-1 — by more than 65 times, the dihydrolipoyltransacetylase component of the pyruvate dehydrogenase complex — by 65 times, transketolase — by 50 times, dehydrogenase/reductase 4 of the SDR family — by almost 100 times, peroxisomal trans-2-enoyl-coenzyme A reductase — by 115 times, villin — by more than 270 times, vitamin D-binding protein — by 84 times, mitochondrial aconitate hydratase — by 45 times, acyl-CoA dehydrogenase of very long chain fatty acids — by almost 44 times, small nuclear ribonucleoprotein Sm D3 — by 46 times (Table 2, Fig. 2). The relative content of various T-complex subunits increases by 20–40 times, and the relative content of a number of heat shock proteins increases by 20–50 times (Table 2, Fig. 2).

At the same time, almost all proteins, bound to RP220 peptide fragments and characterized by the altered relative content in SHR compared to control animals, are related (with a few exceptions) to the development of cardiovascular pathology (relevant references are given in Tables 2–5). For example, fructose-1,6-bisphosphatase is one of the regulatory enzymes of gluconeogenesis; its content is increased in metabolic syndrome. Animal models have shown that disturbances in fructose metabolism lead not only to obesity and diabetes, but also to hypertension [23–25].

Interestingly, many ribosomal proteins bound to the RP224-232 peptide in SHR rats. It is known that, in addition to participating in the translation process, ribosomal proteins perform many extra-ribosomal functions associated with proliferation, differentiation, DNA repair, apoptosis and other cellular processes. Dysfunction of ribosomal proteins is associated with the development of oncological, hematological, metabolic and, in particular, cardiovascular diseases [19–22]. Certain evidence exists that the ribosomal protein L17 plays a role in the regulation of proliferation of the carotid artery smooth muscle cells [36].

The relative content of the key antioxidant enzyme catalase, bound to the RP224-232 peptide, demonstrated a more than 230-fold increase in SHR rats. There is evidence that overexpression of catalase in renal proximal tubule cells in a special line of transgenic mice led to normalization of oxidative stress, prevented development of hypertension, normalized the expression level of angiotensin-converting enzyme 2, improved glomerular filtration, prevented albuminuria, renal hypertrophy, tubulointerstitial fibrosis, apoptosis, and suppressed the expression of pro-fibrotic, and pro-apoptotic genes [16].

Among RP224-232 bound proteins, which demonstrated decreased relative content in SHR rats, the most pronounced (250-fold) relative decrease was found in the case of stratifin (Table 3, Fig. 3). A significant decrease in the relative content was also found for annexins A1 and A2, myosin heavy chain 9, calnexin, tubulin beta 5 chain, and elongation factor 2 bound (by 48, 12, 19, 6, 6, and 8 times, respectively) (Table 3, Fig. 3).

Stratifin (also known as 14-3-3 sigma) belongs to the 14-3-3 protein family; proteins of this family participate in the regulation of numerous intracellular processes. Increased levels of stratifin were detected in endothelial cells of patients and in a strain of rats with pulmonary hypertension. Targets of signaling pathways, activated with the participation of this protein, are intensively studied [50]. Annexin A1 is a glucocorticoid-regulated protein with anti-inflammatory and regulatory effects; it prevents age-related changes in the cardiovascular system and regulates blood pressure. Mice lacking the annexin A1 gene demonstrate structural and functional cardiovascular disorders: high blood pressure, impaired cardiac activity, cardiac hypertrophy, marked vascular remodeling, inflammation, and premature aging [38]. Myosin 9 is a cellular myosin that plays a role in cytokinesis, cytoskeletal reorganization, secretion and other cellular processes. A correlation has been found between hypertension and the myosin heavy chain 9 gene polymorphism in patients with chronic kidney disease in China [49].

Tables 2 and 3 show that in the case of the RP224-232 peptide (a fragment of the RP220 peptide) in almost all cases the fold change in the relative protein content in hypertensive rats compared to control animals exceeded that for the original RP220 peptide. No such changes were found during comparison of proteins bound to the RP233-239 fragment and to the parent RP220 peptide (Tables 4 and 5).

The relative content of catalase, glutathione-S-transferase alpha-1, dihydrolipoyltransacetylase component of the pyruvate dehydrogenase complex in SHR rats increased compared to the controls: in the case of RP224-232 peptide by 234 times, 65 times, and 65 times, respectively, while in the case of RP220 peptide no such changes were found. The relative content of fructose-1,6-bisphosphatase 1 bound to RP224-232 peptide increased in SHR rats compared to the controls by almost 2247 times, and in the case of RP220 peptide the increase was significantly lower (just by 159 times). In the case of 40S ribosomal protein S20, this difference was almost 65 times, while in the case of transketolase more than 11 times (Table 2). The same can be said for kidney proteins, characterized by decreased relative content in SHR compared to the control rats. In the case of the RP220 peptide, this decrease was much less noticeable for almost all proteins than in the case of proteins bound to its RP224-232 fragment (Table 3).

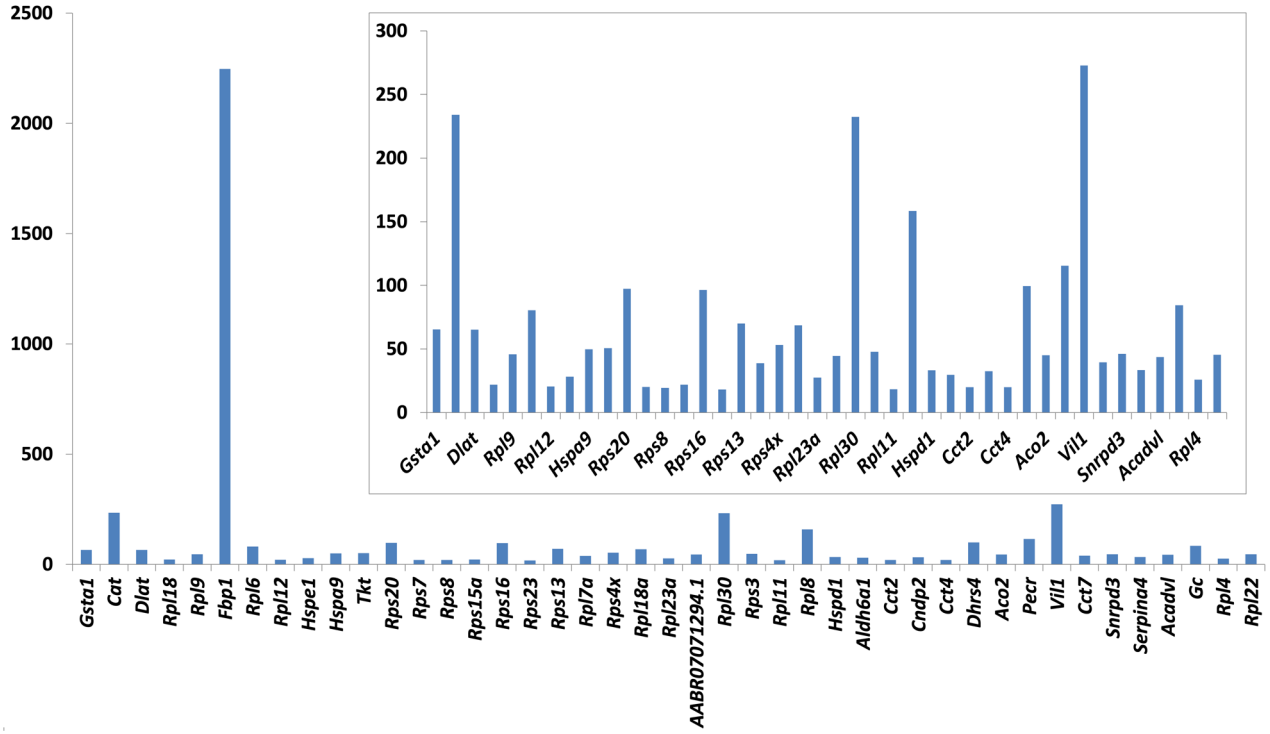

Figure 2. SHR kidney proteins bound to the RP224-232 peptide and demonstrating increased relative content compared to normotensive WKY rats (genes encoding the proteins characterized by the most pronounced increase are indicated). The inset shows the same without fructose-1,6-bisphosphatase 1.

Table 2. SHR kidney proteins bound to RP224-232 and demonstrating at least 18-fold increase in the relative content compared to that of normotensive WKY rats

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Fold change increase		Role in cardio-vascular pathology
						RP224-232	RP220	
1	P00502	*Gsta1*	Glutathione S-transferase alpha-1	4	C	65.325	—	—
2	P04762	*Cat*	Catalase	4	P, MCh, EPR	233.931	—	[16]
3	P08461	*Dlat*	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	1	MCh	65.208	—	[17, 18]
4	P12001	*Rpl18*	60S ribosomal protein L18	5	C, EPR	21.946	4.708	[19–22]
5	P17077	*Rpl9*	60S ribosomal protein L9	5	C	45.834	2.780	[19–22]
6	P19112	*Fbp1*	Fructose-1,6-bisphosphatase 1	1	C	2246.794	159.786	[23–25]
7	P21533	*Rpl6*	60S ribosomal protein L6	5	C, EPR	80.472	10.585	[19–22]
8	B2RYU2	*Rpl12*	60S ribosomal protein L12	5	C	20.468	2.842	[19–22]
9	P26772	*Hspe1*	10 kDa heat shock protein, mitochondrial	4	MCh	28.237	2.487	[26]
10	P48721	*Hspa9*	Stress-70 protein. mitochondrial	4	MCh, N	49.776	6.783	[27]
11	P50137	*Tkt*	Transketolase	1	C	50.538	4.387	[28]
12	A0A0H2UHG7	*Rps20*	40S ribosomal protein S20	5	C, N	97.196	1.552	[19–22]
13	B5DEL9	*Rps7*	40S ribosomal protein S7	5	C	20.036	0.254	[19–22]
14	B2RYR8	*Rps8*	40S ribosomal protein S8	5	C	19.428	—	[19–22]
15	P62246	*Rps15a*	40S ribosomal protein S15a	5	C	21.890	1.697	[19–22]
16	P62250	*Rps16*	40S ribosomal protein S16	5	C	96.403	3.646	[19–22]
17	P62268	*Rps23*	40S ribosomal protein S23	5	C, EPR	18.155	—	[19–22]

Table 2. SHR kidney proteins bound to RP224-232 and demonstrating at least 18-fold increase in the relative content compared to that of normotensive WKY rats (continued)

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Fold change increase		Role in cardio-vascular pathology
						RP224-232	RP220	
18	P62278	*Rps13*	40S ribosomal protein S13	5	C	70.031	1.998	[19–22]
19	F1M013	*Rpl7a*	60S ribosomal protein L7a	5	C	38.699	4.383	[19–22]
20	X1WI37	*Rps4x*	40S ribosomal protein S4	5	C	53.085	—	[19–22]
21	P62718	*Rpl18a*	60S ribosomal protein L18a	5	C	68.526	9.815	[19–22]
22	P62752	*Rpl23a*	60S ribosomal protein L23a	5	C, N	27.412	—	[19–22]
23	F1M6F4	*AABR07071294.1*	40S ribosomal protein S25	5	C	44.576	—	[19–22]
24	P62890	*Rpl30*	60S ribosomal protein L30	5	C	232.335	—	[19–22]
25	P62909	*Rps3*	40S ribosomal protein S3	5	C, M, MCh, N	47.671	1.557	[19–22]
26	P62914	*Rpl11*	60S ribosomal protein L11	5	C, N	18.267	1.141	[19–22]
27	P62919	*Rpl8*	60S ribosomal protein L8	5	C	158.494	—	[19–22]
28	P63039	*Hspd1*	60 kDa heat shock protein, mitochondrial	4	MCh	33.250	—	[29]
29	G3V7J0	*Aldh6a1*	Aldehyde dehydrogenase 6 family, member A1	6	MCh, N	29.684	9.952	[30]
30	Q5XIM9	*Cct2*	T-complex protein 1 subunit beta	4	C	19.979	0.403	—
31	Q6Q0N1	*Cndp2*	Cytosolic non-specific dipeptidase	6	C	32.478	—	—
32	Q7TPB1	*Cct4*	T-complex protein 1 subunit delta	4	C	19.860	2.344	[30]
33	Q8VID1	*Dhrs4*	Dehydrogenase/reductase SDR family member 4	7	P	99.405	—	—
34	Q9ER34	*Aco2*	Aconitate hydratase, mitochondrial	1	MCh	44.971	4.512	[31]
35	Q9WVK3	*Pecr*	Peroxisomal trans-2-enoyl-CoA reductase	7	P	115.331	—	—
36	B5DFA0	*Vil1*	Vil1 protein	2	C	272.801	144.387	—
37	D4AC23	*Cct7*	T-complex protein 1 subunit eta	4	C	39.470	—	[30]
38	M0R907	*Snrpd3*	Small nuclear ribonucleoprotein Sm D3	5	C, N	46.080	—	—
39	Q5M8C3	*Serpina4*	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	3	C	33.328	—	[32, 33]
40	P45953	*Acadvl*	Very long-chain specific acyl-CoA dehydrogenase, mitochondrial	7	MCh	43.635	—	[34]
41	P04276	*Gc*	Vitamin D-binding protein	2	S	84.305	—	[35]
42	Q6P3V9	*Rpl4*	60S ribosomal protein L4	5	C	25.888	—	[19–22]
43	P47198	*Rpl22*	60S ribosomal protein L22	5	C	45.322	2.552	[19–22]

Here and in Tables 3–5, the numbers in the "functions" column designate the following functional groups of proteins: *1.* Proteins/enzymes involved in energy generation and carbohydrate metabolism. *2.* Proteins involved in cytoskeleton formation, transport, and exocytosis. *3.* Proteins involved in signal transduction and regulation of enzyme activity. *4*. Antioxidant and protective proteins/enzymes. *5.* Proteins regulating gene expression, cell division, and differentiation. *6.* Enzymes involved in the metabolism of proteins, amino acids, and other nitrogenous compounds. *7.* Enzymes involved in lipid metabolism.

Protein localization: C – cytoplasm, N – nucleus, M – membranes, PM – plasma membrane, MCh – mitochondria, EPR – endoplasmic reticulum, Ve – vesicles, P – peroxisomes, S – secreted proteins.

Here and in Tables 3–5, the data on the change in the relative content of proteins in the kidneys of hypertensive animals (compared to that of control animals) bound to RP220 peptide are taken from [10].

For the convenience of searching, protein names are given in the form in which they appear in the Uniprot database.

The statistical value -LOG(P-value) for parameters characterizing relative content of proteins bound to RP224-232 was within the range 2.13–6.44; more details are given in Table S1 (Supplementary Materials).

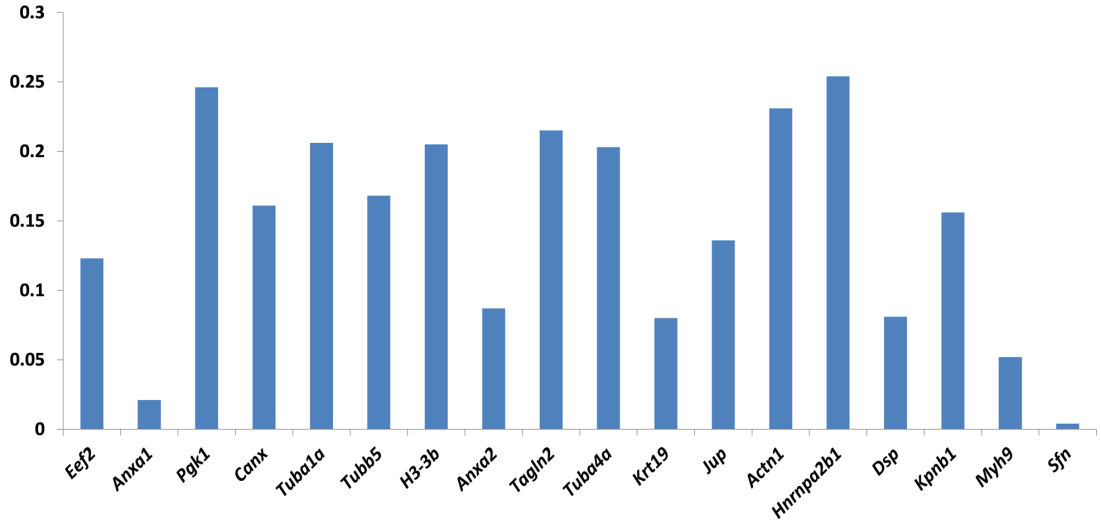

Figure 3. SHR kidney proteins bound to the RP224-232 peptide and demonstrating decreased relative content compared to that of normotensive WKY rats (genes encoding the proteins characterized by the most pronounced decrease are indicated).

Table 3. SHR kidney proteins bound to RP224-232 and demonstrating at least 4-fold decrease in the relative content compared to that of normotensive WKY rats

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Fold change decrease		Role in cardio-vascular pathology
						RP224-232	RP220	
1	P05197	*Eef2*	Elongation factor 2	5	C, N	0.123	0.240	[37]
2	P07150	*Anxa1*	Annexin A1	2	PM, C	0.021	0.237	[38]
3	P16617	*Pgk1*	Phosphoglycerate kinase 1	1	C	0.246	0.466	[39]
4	P35565	*Canx*	Calnexin	4	EPR	0.161	0.353	[40]
5	P68370	*Tuba1a*	Tubulin alpha-1A chain	2	C, MCh	0.206	—	[41]
6	P69897	*Tubb5*	Tubulin beta-5 chain	2	C	0.168	—	[41]
7	P84245	*H3-3b*	Histone H3.3	5	N	0.205	0.296	[42]
8	Q07936	*Anxa2*	Annexin A2	2	PM, C	0.087	0.323	[43]
9	Q5XFX0	*Tagln2*	Transgelin-2	2	C	0.215	0.292	[44]
10	Q5XIF6	*Tuba4a*	Tubulin alpha-4A chain	2	C	0.203	0.338	[41]
11	Q63279	*Krt19*	Keratin, type I cytoskeletal 19	2	C	0.080	—	—
12	Q6P0K8	*Jup*	Junction plakoglobin	2	C, M	0.136	1.989	[45]
13	Q9Z1P2	*Actn1*	Alpha-actinin-1	2	C, M, PM	0.231	—	[46]
14	A7VJC2	*Hnrnpa2b1*	Heterogeneous nuclear ribonucleoproteins A2/B1	5	C, N, S	0.254	—	[47]
15	F1LMV6	*Dsp*	Desmoplakin	2	PM, M	0.081	—	[48]
16	P52296	*Kpnb1*	Importin subunit beta-1	3	C, N	0.156	0.128	—
17	Q62812	*Myh9*	Myosin-9	2	C, Ve	0.052	—	[49]
18	G3V9A3	*Sfn*	Stratifin	5	C, N	0.004	—	[50]

The statistical value -LOG(P-value) for parameters characterizing relative content of proteins bound to RP224-232 was within the range 1.67–6.44; more details are given in Table S2 (Supplementary Materials).

The changes in the relative content of SHR kidney proteins, bound to RP233-239, were not as obvious as for the RP224-232 bound protein. The most pronounced increase in the relative content was observed for fructose-1,6-bisphosphatase 1 (almost 43 times), catalase (21 times), medium- and long-chain 2-hydroxymonocarboxylic acid oxidase (13 times), argininosuccinate synthase (11 times), and transketolase (9 times) (Table 4, Fig. 4). The decrease in the relative content of SHR kidney proteins, bound to RP233-239, was insignificant (less than 4 times compared to the control) (Table 5, Fig. 5).

Table 4. SHR kidney proteins bound to RP233-239 and demonstrating at least 2-fold increase in the relative content compared to that of normotensive WKY rats

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Fold change increase		Role in cardio-vascular pathology
						RP233-239	RP220	
1	O70351	*Hsd17b10*	3-hydroxyacyl-CoA dehydrogenase type-2	7	MCh	4.919	—	—
2	P02761	*Mup*	Major urinary protein	3	C, S	8.589	19.433	[51]
3	P04762	*Cat*	Catalase	4	P, C	21.061	—	[16]
4	P05065	*Aldoa*	Fructose-bisphosphate aldolase A	1	C	6.666	—	[52]
5	Q6LDS4	*Sod1*	Superoxide dismutase [Cu-Zn]	4	C, N	6.793	—	[53, 54]
6	P07756	*Cps1*	Carbamoyl-phosphate synthase [ammonia], mitochondrial	6	MCh, N	8.299	—	[55]
7	P08461	*Dlat*	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	1	MCh	8.805	—	[18]
8	P09034	*Ass1*	Argininosuccinate synthase	6	C	11.096	12.192	[56]
9	P17077	*Rpl9*	60S ribosomal protein L9	5	C	4.488	2.780	[19, 20]
10	P17764	*Acat1*	Acetyl-CoA acetyltransferase, mitochondria	7	MCh	2.348	—	[57]
11	P19112	*Fbp1*	Fructose-1,6-bisphosphatase 1	1	C	42.678	159.814	[58]
12	P49242	*Rps3a*	40S ribosomal protein S3a	5	C, N	3.639	—	[19, 20]
13	P50137	*Tkt*	Transketolase	1	C	9.717	4.387	—
14	P62271	*Rps18*	40S ribosomal protein S18	5	C	4.293	—	[19–22]
15	P62718	*Rpl18a*	60S ribosomal protein L18a	5	C	8.270	9.817	[19–22]
16	P62755	*Rps6*	40S ribosomal protein S6	5	C, N	4.647	—	[19–22]
17	P98158	*Lrp2*	Low-density lipoprotein receptor-related protein 2	3	PM, EPR, M	5.253	117.621	[59–61]
18	Q07523	*Hao2*	2-Hydroxyacid oxidase 2	7	P	13.392	202.39	[62]
19	Q60587	*Hadhb*	Trifunctional enzyme subunit beta, mitochondrial	7	MCh	4.854	9.666	[63]
20	Q63716	*Prdx1*	Peroxiredoxin-1	4	C	5.015	10.14	[64–66]
21	Q9ER34	*Aco2*	Aconitate hydratase, mitochondrial	1	MCh	4.510	4.512	[31]
22	Q9Z0V6	*Prdx3*	Thioredoxin-dependent peroxide reductase, mitochondrial	4	C, EPR, MCh	5.577	—	—
23	D4ACB8	*Cct8*	T-complex protein 1 subunit theta	4	C, MCh	4.536	2.294	—
24	P20760	*Igg-2a*	Ig gamma-2A chain C region	4	C	4.468	—	—
25	Q6P3V9	*Rpl4*	60S ribosomal protein L4	5	C	6.506	—	[19–22]

The statistical value -LOG(P-value) for parameters characterizing relative content of proteins bound to RP233-239 was within the range 1.11–4.29; more details are given in Table S3 (Supplementary Materials).

CONCLUSIONS

RP220 is a 20-membered peptide that corresponds to a fragment of the RNLS amino acid sequence, including residues 220-239, and reproduces a number of RNLS effects [5, 8]. It contains several sites for potential cleavage by extracellular and circulating proteases, which can apparently form several peptides, including RP224-232 and RP233-239 [7, 9].

In this study, we have demonstrated that the change in the relative content of kidney proteins, bound to the studied RNLS peptides in hypertensive (SHR) rats, compared to that in normotensive (WKY) animals, is most clearly manifested in the case of the RP224-232 peptide as an affinity ligand. Many of these RP224-232 bound proteins are associated with cardiovascular pathology, including hypertension. This suggests that proteolytic processing of RP220 does not result in the inactivation of this peptide, but does result in changes of ligand/regulatory properties, as well as the repertoire of potential protein partners and, therefore, protein-protein interactions that may have potential pharmacological applications.

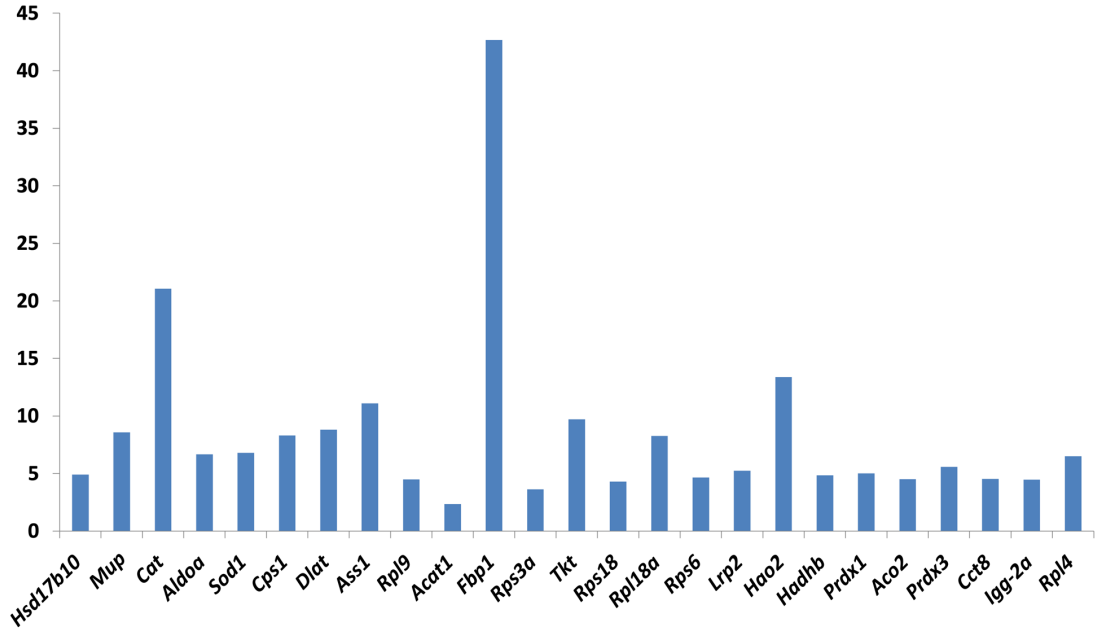

Figure 4. SHR kidney proteins bound to the RP233-239 peptide and demonstrating increased relative content compared to that of normotensive WKY rats (genes encoding the proteins characterized by the most pronounced increase are indicated).

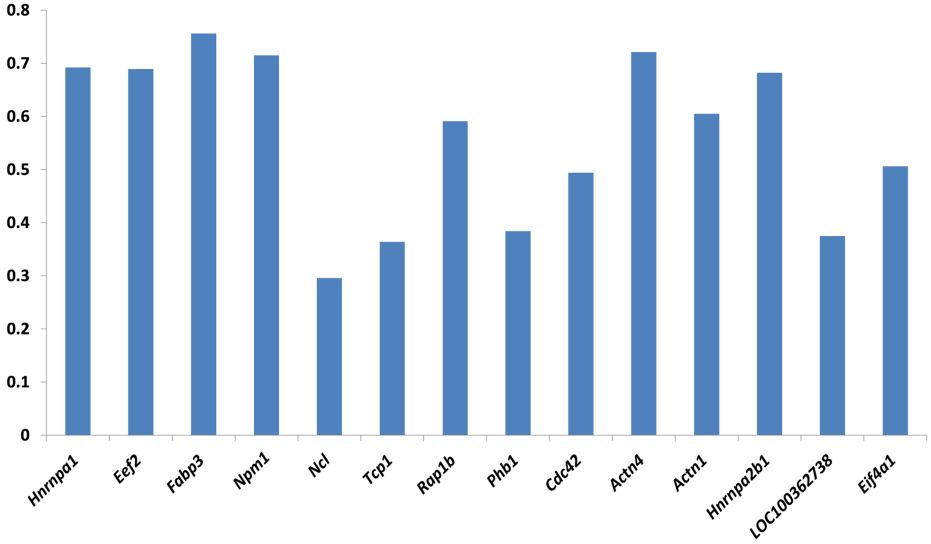

Figure 5. SHR kidney proteins bound to the RP233-239 peptide and demonstrating decreased relative content compared to that of normotensive WKY rats (genes encoding the proteins characterized by the most pronounced decrease are indicated).

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Mass spectrometry analysis of proteins was carried out using the equipment of the "Human Proteome" Core Facility (IBMC).

COMPLIANCE WITH ETHICAL STANDARDS

The experiments were carried out in compliance with generally accepted norms of humane treatment of laboratory animals, in accordance with the Order of the Ministry of Health of the Russian Federation No. 199n of April 1, 2016 "On Approval of the Rules for Good Laboratory Practice".

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

Supplementary materials are available in the electronic version at the journal site (pbmc.ibmc.msk.ru).

Table 5. SHR kidney proteins bound to RP233-239 and demonstrating a decrease in the relative content compared to that of normotensive WKY rats

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	Fold change decrease		Role in cardio-vascular pathology
						RP233-239	RP220	
1	P04256	*Hnrnpa1*	Heterogeneous nuclear ribonucleoprotein A1	5	C, N, S	0.692	0.262	[67]
2	P05197	*Eef2*	Elongation factor 2	5	C, N	0.689	0.239	[37]
3	P07483	*Fabp3*	Fatty acid-binding protein, heart	7	C	0.756	—	[68]
4	P13084	*Npm1*	Nucleophosmin	5	N, C, MCh	0.715	0.430	[69]
5	P13383	*Ncl*	Nucleolin	5	C, N	0.296	—	[70]
6	P28480	*Tcp1*	T-complex protein 1 subunit alpha	4	C, MCh	0.364	—	[30]
7	Q62636	*Rap1b*	Ras-related protein Rap-1b	3	PM, C, M	0.591	0.361	[71]
8	P67779	*Phb1*	Prohibitin 1	3	PM, M, MCh, N	0.384	0.282	[72]
9	Q8CFN2	*Cdc42*	Cell division control protein 42 homolog	2	C, M, PM	0.494	0.399	[73]
10	Q9QXQ0	*Actn4*	Alpha-actinin-4	2	C, N	0.721	0.589	[46]
11	Q9Z1P2	*Actn1*	Alpha-actinin-1	2	C, M, PM	0.605	0.472	[46]
12	A7VJC2	*Hnrnpa2b1*	Heterogeneous nuclear ribonucleoproteins A2/B1	5	C, N, S	0.682	0.283	[47]
13	M0RD14	*LOC100362738*	Pyruvate kinase	1	C	0.375	0.537	—
14	Q6P3V8	*Eif4a1*	ATP-dependent RNA helicase	5	C	0.506	0.071	—

The statistical value -LOG(P-value) for parameters characterizing relative content of proteins bound to RP233-239 was within the range 1.00–3.56; more details are given in Table S4(Supplementary Materials).

REFERENCES

1. *Xu J., Li G., Wang P., Velazquez H., Yao X., Li Y., Wu Y., Peixoto A., Crowley S., Desir G.V.* (2005) Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. J. Clin. Invest., **115**(5), 1275–1280. DOI: 10.1172/JCI24066
2. *Medvedev A.E., Veselovsky A.V., Fedchenko V.I.* (2010) Renalase, a new secretory enzyme responsible for selective degradation of catecholamines: achievements and unsolved problems. Biochemistry (Moscow), **75**(8), 951–958. DOI: 10.1134/s0006297910080018
3. *Desir G.V., Peixoto A.J.* (2014) Renalase in hypertension and kidney disease. Nephrol. Dial. Transplant., **29**(1), 22–28. DOI: 10.1093/ndt/gft083
4. *Wang Y., Safirstein R., Velazquez H., Guo X.-J., Hollander L., Chang J., Chen T.-M., Mu J.-J., Desir G.V.* (2017) Extracellular renalase protects cells and organs by outside-in signalling. J. Cell. Mol. Med., **21**(7), 1260–1265. DOI: 10.1111/jcmm.13062
5. *Pointer T.C., Gorelick F.S., Desir G.V.* (2021) Renalase: a multi-functional signaling molecule with roles in gastrointestinal disease. Cells, **10**(8), 2006. DOI: 10.3390/cells10082006
6. *Moran G.R., Hoag M.R.* (2017) The enzyme: renalase. Arch. Biochem. Biophys., **632**, 66–76. DOI: 10.1016/j.abb.2017.05.015
7. *Fedchenko V.I., Veselovsky A.V., Kopylov A.T., Kaloshina S.A., Medvedev A.E.* (2022) Renalase may be cleaved in blood. Are blood chymotrypsin-like enzymes involved? Medical Hypotheses, **165**, 110895. DOI: 10.1016/j.mehy.2022.110895
8. *Kolodecik T.R., Guo X., Shugrue C.A., Guo X., Desir G.V., Wen L., Gorelick F.* (2024) Renalase peptides reduce pancreatitis severity in mice. Am. J. Physiol. Gastrointest. Liver Physiol., **327**(3), G466–G480. DOI: 10.1152/ajpgi.00143.2024
9. *Buneeva O.A., Fedchenko V.I., Gnedenko O.V., Kaloshina S.A., Medvedeva M.V., Zavyalova M.G., Ivanov A.S., Zgoda V.G., Medvedev A.E.* (2025) Interaction of rat kidney proteins with the renalase peptide RP220 and its potential proteolytic fragment RP224-232: a comparative proteomic analysis. Biomeditsinskaya Khimiya, **71**(1), 65–70. DOI: 10.18097/PBMCR1559
10. *Buneeva O.A., Fedchenko V.I., Kaloshina S.A., Zavyalova M.G, Zgoda V.G., Medvedev A.E.* (2024) Proteomic profiling of renal tissue of normo- and hypertensive rats with the renalase peptide RP220 as an affinity ligand. Biomeditsinskaya Khimiya, **70**(3), 145–155. DOI: 10.18097/PBMC20247003145
11. *Bradford M.M.* (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., **72**, 248–254. DOI: 10.1016/0003-2697(76)90527-3
12. *Buneeva O.A., Kopylov A.T., Gnedenko O.V., Medvedeva M.V., Kapitsa I.G, Ivanova E.A., Ivanov A.S., Medvedev A.E.* (2021) Changes in the mitochondrial subproteome of mouse brain Rpn13-binding proteins induced by the neurotoxin MPTP and the neuroprotector isatin. Biomeditsinskaya Khimiya, **67**(1), 51–65. DOI: 10.18097/PBMC20216701051
13. *Akins R., Tuan R.* (1995) Ultrafast Protein Determinations Using Microwave Enhancement. In: The Protein Protocols Handbook (Walker J.M., ed.). Springer Protocols Handbooks. Humana Press, pp. 23–30. DOI: 10.1385/1-59259-169-8:23

14. *Wiśniewski J.R., Zougman A., Nagaraj N., Mann M.* (2009) Universal sample preparation method for proteome analysis. Nat. Methods, **6**(5), 359–362. DOI: 10.1038/nmeth.1322
15. *Buneeva O.A., Fedchenko V.I., Kaloshina S.A., Zavyalova M.G., Zgoda V.G., Medvedev A.E.* (2024) Comparative proteomic analysis of renal tissue of normotensive and hypertensive rats. Biomeditsinskaya Khimiya, **70**(2), 89–98. DOI: 10.18097/PBMC20247002089
16. *Shi Y., Lo C.-S., Chenier I., Maachi H., Filep J.G., Ingelfinger J.R., Zhang S.-L., Chan J.S.D.* (2013) Overexpression of catalase prevents hypertension and tubulointerstitial fibrosis and normalization of renal angiotensin-converting enzyme-2 expression in Akita mice. Am. J. Physiol. Renal Physiol., **304**(11), F1335–F1346. DOI: 10.1152/ajprenal.00405.2012
17. *Wang L., Hou E., Wang Z., Sun N., He L., Chen L., Liang M., Tian Z.* (2014) Analysis of metabolites in plasma reveals distinct metabolic features between Dahl salt-sensitive rats and consomic SS.13(BN) rats. Biochem. Biophys. Res. Commun., **450**(1), 863–869. DOI: 10.1016/j.bbrc.2014.06.089
18. *Quinonez S.C., Thoene J.G.* (1993) Dihydrolipoamide Dehydrogenase Deficiency. In: GeneReviews® (Adam M.P., Feldman J., Mirzaa G.M., Pagon R.A., Wallace S.E., Amemiya A., eds.). University of Washington, Seattle, Seattle (WA).
19. *Kang J., Brajanovski N., Chan K.T., Xuan J., Pearson R.B., Sanij E.* (2021) Ribosomal proteins and human diseases: molecular mechanisms and targeted therapy. Signal Transduct. Target. Ther., **6**, 323. DOI: 10.1038/s41392-021-00728-8
20. *Bhavsar R.B., Makley L.N., Tsonis P.A.* (2010) The other lives of ribosomal proteins. Hum. Genomics, **4**(5), 327–344. DOI: 10.1186/1479-7364-4-5-327
21. *Zhou X., Liao W.-J., Liao J.-M., Liao P., Lu H.* (2015) Ribosomal proteins: functions beyond the ribosome. J. Mol. Cell Biol., **7**(2), 92–104. DOI: 10.1093/jmcb/mjv014
22. *Wang W., Nag S., Zhang X., Wang M.-H., Wang H., Zhou J., Zhang R.* (2015) Ribosomal proteins and human diseases: pathogenesis, molecular mechanisms, and therapeutic implications. Med. Res. Rev., **35**(2), 225–285. DOI: 10.1002/med.21327
23. *Visinoni S., Khalid N.F.I., Joannides C.N., Shulkes A., Yim M., Whitehead J., Tiganis T., Lamont B.J., Favaloro J.M., Proietto J., Andrikopoulos S., Fam B.C.* (2012) The role of liver fructose-1,6-bisphosphatase in regulating appetite and adiposity. Diabetes, **61**(5), 1122–1132. DOI: 10.2337/db11-1511
24. *Polak-Iwaniuk A., Harasim-Symbor E., Gołaszewska K., Chabowski A.* (2019) How hypertension affects heart metabolism. Front. Physiol., **10**, 435. DOI: 10.3389/fphys.2019.00435
25. *Khitan Z., Kim D.H.* (2013) Fructose: a key factor in the development of metabolic syndrome and hypertension. J. Nutr. Metab., **2013**, 682673. DOI: 10.1155/2013/682673
26. *Maciel L., de Oliveira D.F., Monnerat G., Campos de Carvalho A.C., Nascimento J.H.M.* (2020) Exogenous 10 kDa-heat shock protein preserves mitochondrial function after hypoxia/reoxygenation. Front. Pharmacol., **11**, 545. DOI: 10.3389/fphar.2020.00545
27. *Lopez-Campistrous A., Hao L., Xiang W., Ton D., Semchuk P., Sander J., Ellison M.J., Fernandez-Patron C.* (2008) Mitochondrial dysfunction in the hypertensive rat brain: respiratory complexes exhibit assembly defects in hypertension. Hypertension, **51**(2), 412–419. DOI: 10.1161/HYPERTENSIONAHA.107.102285
28. *Wang C., Zhang L., Zhang Q., Zheng H., Yang X., Cai W., Zou Q., Lin J., Zhang L., Zhong L., Li X., Liao Y., Liu Q., Chen L., Li Y.* (2024) Transketolase drives the development of aortic dissection by impairing mitochondrial bioenergetics. Acta Physiol, **240**(4), e14113. DOI: 10.1111/apha.14113
29. *Duan Y., Tang H., Mitchell-Silbaugh K., Fang X., Han Z., Ouyang K.* (2020) Heat shock protein 60 in cardiovascular physiology and diseases. Front. Mol. Biosci., **7**, 73. DOI: 10.3389/fmolb.2020.00073
30. *Lee M.-J., Stephenson D.A., Groves M.J., Sweeney M.G., Davis M.B., An S.-F., Houlden H., Salih M.A.M., Timmerman V., de Jonghe P., Auer-Grumbach M., di Maria E., Scaravilli F., Wood N.W., Reilly M.M.* (2003) Hereditary sensory neuropathy is caused by a mutation in the delta subunit of the cytosolic chaperonin-containing t-complex peptide-1 (Cct4) gene. Hum. Mol. Genet., **12**(15), 1917–1925. DOI: 10.1093/hmg/ddg198
31. *Dikalov S.I., Ungvari Z.* (2013) Role of mitochondrial oxidative stress in hypertension. Am. J. Physiol. Heart Circ. Physiol., **305**(10), H1417–H1427. DOI: 10.1152/ajpheart.00089.2013
32. *Shashidhar K.N., Lakshmaiah V., Muninarayana C., Nallagangula K.S.* (2021) Quantitative ELISA for SERPINA4/kallistatin. BioTechniques, **71**(5), 556–565. DOI: 10.2144/btn-2018-0194
33. *Krishna S.M., Li J., Wang Y., Moran C.S., Trollope A., Huynh P., Jose R., Biros E., Ma J., Golledge J.* (2021) Kallistatin limits abdominal aortic aneurysm by attenuating generation of reactive oxygen species and apoptosis. Sci. Rep., **11**, 17451. DOI: 10.1038/s41598-021-97042-8
34. *Cox K.B., Liu J., Tian L., Barnes S., Yang Q., Wood P.A.* (2009) Cardiac hypertrophy in mice with long-chain acyl-CoA dehydrogenase or very long-chain acyl-CoA dehydrogenase deficiency. Lab. Invest, **89**(12), 1348–1354. DOI: 10.1038/labinvest.2009.86
35. *Jeong H.Y., Park K.M., Lee M.J., Yang D.H., Kim S.H., Lee S.-Y.* (2017) Vitamin D and hypertension. Electrolyte Blood Press., **15**(1), 1–11. DOI: 10.5049/EBP.2017.15.1.1
36. *Smolock E.M., Korshunov V.A., Glazko G., Qiu X., Gerloff J., Berk B.C.* (2012) Ribosomal protein L17, RpL17, is an inhibitor of vascular smooth muscle growth and carotid intima formation. Circulation, **126**(20), 2418–2427. DOI: 10.1161/CIRCULATIONAHA.112.125971
37. *Liu R., Proud C.G.* (2016) Eukaryotic elongation factor 2 kinase as a drug target in cancer, and in cardiovascular and neurodegenerative diseases. Acta Pharmacol. Sin., **37**(3), 285–294. DOI: 10.1038/aps.2015.123
38. *Singh J., Jackson K.L., Tang F.S., Fu T., Nowell C., Salimova E., Kiriazis H., Ritchie R.H., Head G.A., Woodman O.L., Qin C.X.* (2024) The pro-resolving mediator, annexin A1 regulates blood pressure, and age-associated changes in cardiovascular function and remodeling. FASEB J., **38**(3), e23457. DOI: 10.1096/fj.202301802R
39. *Pan C.-H., Chien Y.-C., Sung M.-S., Huang H.-Y., Sheu M.-J., Wu C.-H.* (2021) Pathological role of phosphoglycerate kinase 1 in balloon angioplasty-induced neointima formation. Int. J. Mol. Sci., **22**(16), 8822. DOI: 10.3390/ijms22168822
40. *Balhara M., Neikirk K., Marshall A., Hinton A. Jr., Kirabo A.* (2024) Endoplasmic reticulum stress in hypertension and salt sensitivity of blood pressure. Curr. Hypertens. Rep., **26**(6), 273–290. DOI: 10.1007/s11906-024-01300-9
41. *Shi X., Jiang X., Chen C., Zhang Y., Sun X.* (2022) The interconnections between the microtubules and mitochondrial networks in cardiocerebrovascular diseases: implications for therapy. Pharmacol. Res., **184**, 106452. DOI: 10.1016/j.phrs.2022.106452

42. *Goldman J.A., Kuzu G., Lee N., Karasik J., Gemberling M., Foglia M.J., Karra R., Dickson A.L., Sun F., Tolstorukov M.Y., Poss K.D.* (2017) Resolving heart regeneration by replacement histone profiling. Dev. Cell, **40**(4), 392–404.e5. DOI: 10.1016/j.devcel.2017.01.013
43. *Pascal A., Gallaud E., Giet R., Benaud C.* (2022) Annexin A2 and Ahnak control cortical NuMA-dynein localization and mitotic spindle orientation. J. Cell Sci., **135**(9), jcs259344. DOI: 10.1242/jcs.259344
44. *Szelenberger R., Jóźwiak P., Kacprzak M., Bijak M., Zielińska M., Olender A., Saluk-Bijak J.* (2022) Variations in blood platelet proteome and transcriptome revealed altered expression of transgelin-2 in acute coronary syndrome patients. Int. J. Mol. Sci., **23**(11), 6340. DOI: 10.3390/ijms23116340
45. *Li J., Swope D., Raess N., Cheng L., Muller E.J., Radice G.L.* (2011) Cardiac tissue-restricted deletion of plakoglobin results in progressive cardiomyopathy and activation of β-catenin signaling. Mol. Cell. Biol., **31**(6), 1134–1144. DOI: 10.1128/MCB.01025-10
46. *Noureddine M., Mikolajek H., Morgan N.V., Denning C., Loughna S., Gehmlich K., Mohammed F.* (2025) Structural and functional insights into α-actinin isoforms and their implications in cardiovascular disease. J. Gen. Physiol., **157**(2), e202413684. DOI: 10.1085/jgp.202413684
47. *Li X., Shi S., Li Z., Wang Y., Qi X., Zhang R., Liu Z., Cheng Y.* (2024) Heterogeneous nuclear ribonucleoprotein A2/B1 promotes myocardial fibrosis by regulating the miR-221-3p/FOXO4-mediated inflammation. Clin. Transl. Med., **14**(3), e1616. DOI: 10.1002/ctm2.1616
48. *Brandão M., Bariani R., Rigato I., Bauce B.* (2023) Desmoplakin cardiomyopathy: comprehensive review of an increasingly recognized entity. J. Clin. Med., **12**(7), 2660. DOI: 10.3390/jcm12072660
49. *Liu L., Wang C., Mi Y., Liu D., Li L., Fan J., Nan L., Jia N., Du Y.* (2016) Association of MYH9 polymorphisms with hypertension in patients with chronic kidney disease in China. Kidney Blood Press. Res., **41**(6), 956–965. DOI: 10.1159/000452597
50. *James J., Dejesus V., Rafikova O., Rafikov R.* (2024) Abstract 4146556: Stratifin is a critical target in pulmonary hypertension. Circulation, **150**(Suppl_1), A4146556–A4146556. DOI: 10.1161/circ.150.suppl_1.4146556
51. *Gómez-Baena G, Armstrong S.D., Halstead J.O., Prescott M., Roberts S.A., McLean L., Mudge J.M., Hurst J.L., Beynon R.J.* (2019) Molecular complexity of the major urinary protein system of the Norway rat, *Rattus norvegicus*. Sci. Rep., **9**, 10757. DOI: 10.1038/s41598-019-46950-x
52. *Li Y., Zhang D., Kong L., Shi H., Tian X., Gao L., Liu Y., Wu L., Du B., Huang Z., Liang C., Wang Z., Yao R., Zhang Y.* (2018) Aldolase promotes the development of cardiac hypertrophy by targeting AMPK signaling. Exp. Cell Res., **370**(1), 78–86. DOI: 10.1016/j.yexcr.2018.06.009
53. *Sato M., Yanagisawa H., Nojima Y., Tamura J., Wada O.* (2002) Zn deficiency aggravates hypertension in spontaneously hypertensive rats: possible role of Cu/Zn-superoxide dismutase. Clin. Exp. Hypertens., **24**(5), 355–370. DOI: 10.1081/ceh-120004797
54. *Yanagisawa H., Sato M., Nodera M., Wada O.* (2004) Excessive zinc intake elevates systemic blood pressure levels in normotensive rats — potential role of superoxide-induced oxidative stress. J. Hypertens., **22**(3), 543–550. DOI: 10.1097/00004872-200403000-00017
55. *El-Khazragy N., El Barbary M., Fouad H., Abdelgawad A., Rabie D.* (2021) Association between genetic variations in carbamoyl-phosphate synthetase gene and persistent neonatal pulmonary hypertension. Eur. J. Pediatr., **180**(9), 2831–2838. DOI: 10.1007/s00431-021-04053-8
56. *Guerreiro J.R., Lameu C., Oliveira E.F., Klitzke C.F., Melo R.L., Linares E., Augusto O., Fox J.W., Lebrun I., Serrano S.M.T., Camargo A.C.M.* (2009) Argininosuccinate synthetase is a functional target for a snake venom anti-hypertensive peptide: role in arginine and nitric oxide production. J. Biol. Chem., **284**(30), 20022–20033. DOI: 10.1074/jbc.M109.021089
57. *Zhou T., Yang H., Wang H., Luo N., Xia Y., Jiang X.* (2022) Association between ACAT1 rs1044925 and increased hypertension risk in Tongdao Dong. Medicine (Baltimore), **101**(49), e32196. DOI: 10.1097/MD.0000000000032196
58. *Bonnet S., Paulin R.* (2019) Involvement of PFKFB3 in pulmonary arterial hypertension pathogenesis. Is it all about glycolysis? Am. J. Respir. Crit. Care Med., **200**(5), 532–534. DOI: 10.1164/rccm.201902-0394ED
59. *Calvier L., Herz J., Hansmann G.* (2022) Interplay of low-density lipoprotein receptors, LRPs, and lipoproteins in pulmonary hypertension. JACC Basic Transl. Sci., **7**(2), 164–180. DOI: 10.1016/j.jacbts.2021.09.011
60. *Longoni M., Kantarci S., Donnai D., Pober B.R.* (1993) Donnai-Barrow Syndrome. In: GeneReviews® (Adam M.P., Feldman J., Mirzaa G.M., Pagon R.A., Wallace S.E., Amemiya A., eds.). University of Washington, Seattle, Seattle (WA).
61. *Sendra J., Llorente-Cortés V., Costales P., Huesca-Gómez C., Badimon L.* (2008) Angiotensin II upregulates LDL receptor-related protein (LRP1) expression in the vascular wall: a new pro-atherogenic mechanism of hypertension. Cardiovasc. Res., **78**(3), 581–589. DOI: 10.1093/cvr/cvn043
62. *Barawkar D.A., Meru A., Bandyopadhyay A., Banerjee A., Deshpande A.M., Athare C., Koduru C., Khose G., Gundu J., Mahajan K., Patil P., Kandalkar S.R., Niranjan S., Bhosale S., De S., Mukhopadhyay S., Chaudhary S., Koul S., Singh U., Chugh A., Palle V.P., Mookhtiar K.A., Vacca J., Chakravarty P.K., Nargund R.P., Wright S.D., Roy S., Graziano M.P., Singh S.B., Cully D., Cai T.-Q.* (2011) Potent and selective inhibitors of long chain l-2-hydroxy acid oxidase reduced blood pressure in DOCA salt-treated rats. ACS Med. Chem. Lett., **2**(12), 919–923. DOI: 10.1021/ml2001938
63. *Meng C., Jin X., Xia L., Shen S.-M., Wang X.-L., Cai J., Chen G.-Q., Wang L.-S., Fang N.-Y.* (2009) Alterations of mitochondrial enzymes contribute to cardiac hypertrophy before hypertension development in spontaneously hypertensive rats. J. Proteome Res., **8**(5), 2463–2475. DOI: 10.1021/pr801059u
64. *Jeong S.-J., Park J.-G., Oh G.T.* (2021) Peroxiredoxins as potential targets for cardiovascular disease. Antioxidants (Basel), **10**(8), 1244. DOI: 10.3390/antiox10081244
65. *Jiang L., Gong Y., Hu Y., You Y., Wang J., Zhang Z., Wei Z., Tang C.* (2020) Peroxiredoxin-1 overexpression attenuates doxorubicin-induced cardiotoxicity by inhibiting oxidative stress and cardiomyocyte apoptosis. Oxid. Med. Cell. Longev., **2020**, 2405135. DOI: 10.1155/2020/2405135
66. *Zhou M., Guo J., Li S., Li A., Fang Z., Zhao M., Zhang M., Wang X.* (2023) Effect of peroxiredoxin 1 on the regulation of trophoblast function by affecting autophagy and oxidative stress in preeclampsia. J. Assist. Reprod. Genet., **40**(7), 1573–1587. DOI: 10.1007/s10815-023-02820-0

67. *Zhang L., Chen Q., An W., Yang F., Maguire E.M., Chen D., Zhang C., Wen G., Yang M., Dai B., Luong L.A., Zhu J., Xu Q., Xiao Q.* (2017) Novel pathological role of hnRNPA1 (heterogeneous nuclear ribonucleoprotein A1) in vascular smooth muscle cell function and neointima hyperplasia. Arterioscler. Thromb. Vasc. Biol., **37**(11), 2182–2194. DOI: 10.1161/ATVBAHA.117.310020
68. *Furuhashi M.* (2019) Fatty acid-binding protein 4 in cardiovascular and metabolic diseases. J. Atheroscler. Thromb., **26**(3), 216–232. DOI: 10.5551/jat.48710
69. *Rao C., Liu B., Huang D., Chen R., Huang K., Li F., Dong N.* (2021) Nucleophosmin contributes to vascular inflammation and endothelial dysfunction in atherosclerosis progression. J. Thorac. Cardiovasc. Surg., **161**(5), e377–e393. DOI: 10.1016/j.jtcvs.2019.10.152
70. *Fang L., Shen Z., Zhang Y., Mao Z., Huang D., Lou C.* (2025) Nucleolin in the cell membrane promotes Ang II-mediated VSMC phenotypic switching by regulating the AT1R internalization function: nucleolin promotes Ang II-mediated VSMC phenotypic switching. Biol. Direct, **20**(1), 24. DOI: 10.1186/s13062-025-00615-0
71. *Lakshmikanthan S., Zieba B.J., Ge Z.-D., Momotani K., Zheng X., Lund H., Artamonov M.V., Maas J.E., Szabo A., Zhang D.X., Auchampach J.A., Mattson D.L., Somlyo A.V., Chrzanowska-Wodnicka M.* (2014) Rap1b in smooth muscle and endothelium is required for maintenance of vascular tone and normal blood pressure. Arterioscler. Thromb. Vasc. Biol., **34**(7), 1486–1494. DOI: 10.1161/ATVBAHA.114.303678
72. *Mattox T.A., Psaltis C., Weihbrecht K., Robidoux J., Kilburg-Basnyat B., Murphy M.P., Gowdy K.M., Anderson E.J.* (2021) Prohibitin-1 is a dynamically regulated blood protein with cardioprotective effects in sepsis. J. Am. Heart Assoc., **10**(14), e019877. DOI: 10.1161/JAHA.120.019877
73. *Wu L., Li H., Chen H., Fan C., Lu Y., Wei R., Yang G., Jia Y.* (2023) The clinical utility of circulating cell division control 42 in small-vessel coronary artery disease patients undergoing drug-coated balloon treatment. BMC Cardiovasc. Disord., **23**, 496. DOI: 10.1186/s12872-023-03476-5

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ВЗАИМОДЕЙСТВИЕ БЕЛКОВ ПОЧЕК НОРМО- И ГИПЕРТЕНЗИВНЫХ КРЫС С ФРАГМЕНТАМИ РЕНАЛАЗНОГО ПЕПТИДА RP220

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Реналаза (RNLS) — вовлечённый в регуляцию артериального давления белок, которому свойственны различные функции внутри и снаружи клеток. Двадцатичленный пептид RP220, соответствующий аминокислотной последовательности RNLS человека 220–239, воспроизводит ряд эффектов внеклеточной RNLS и может связываться со многими внутриклеточными белками почек. Последовательность RP220 содержит несколько участков расщепления внеклеточными протеазами, в том числе с образованием пептидов RP224-232 и RP233-239. Целью настоящей работы было протеомное профилирование ткани почек нормотензивных крыс Wistar Kyoto (WKY) и крыс со спонтанной гипертензией SHR (spontaneously hypertensive rats), полученных на основе WKY, с использованием потенциальных протеолитических фрагментов (RP224-232 и RP233-239) пептида RP220 в качестве аффинных лигандов, в сравнении с данными для исходного пептида RP220. Полученные результаты свидетельствуют о том, что относительное содержание связавшихся с RNLS-пептидами белков SHR крыс по сравнению с таковым у крыс WKY наиболее ярко изменяется у пептида RP224-232. Практически все эти белки, за редким исключением, ассоциированы с сердечно-сосудистой патологией, многие с гипертензией. Таким образом, протеолитический процессинг RP220 приводит не к инактивации данного пептида, но к изменению его лигандных/регуляторных свойств, а также репертуара потенциальных белков-партнёров и, следовательно, белок-белковых взаимодействий, которые могут иметь возможное фармакологическое применение.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: реналаза; пептиды реналазы RP220, RP224-232 и RP233-239; артериальная гипертензия; крысы WKY и SHR; протеомное профилирование ткани почек

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