PROTEOMIC PROFILING OF RENAL TISSUE OF NORMO- AND HYPERTENSIVE RATS WITH THE RENALASE PEPTIDE RP220 AS AN AFFINITY LIGAND

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Renalase (RNLS) is a recently discovered protein that plays an important role in the regulation of blood pressure by acting inside and outside cells. Intracellular RNLS is a FAD-dependent oxidoreductase that oxidizes isomeric forms of β-NAD(P)H. Extracellular renalase lacking its N-terminal peptide and cofactor FAD exerts various protective effects via non-catalytic mechanisms. Certain experimental evidence exists in the literature that the RP220 peptide (a 20-mer peptide corresponding to the amino acid sequence RNLS 220–239) reproduces a number of non-catalytic effects of this protein, acting on receptor proteins of the plasma membrane. The possibility of interaction of this peptide with intracellular proteins has not been studied. Taking into consideration the known role of RNLS as a possible antihypertensive factor, the aim of this study was to perform proteomic profiling of the kidneys of normotensive and hypertensive rats using RP220 as an affinity ligand. Proteomic (semi-quantitative) identification revealed changes in the relative content of about 200 individual proteins in the kidneys of hypertensive rats bound to the affinity sorbent as compared to the kidneys of normotensive animals. Increased binding of SHR renal proteins to RP220 over the normotensive control was found for proteins involved in the development of cardiovascular pathology. Decreased binding of the kidney proteins from hypertensive animals to RP220 was noted for components of the ubiquitin-proteasome system, ribosomes, and cytoskeleton.

Key words: renalase; renalase peptide; arterial hypertension; WKY and SHR rats; renal tissue; proteomic profiling

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INTRODUCTION

Renalase (RNLS) is a secretory protein discovered in 2005 that performs various functions inside and outside cells [1-5]. Intracellular RNLS acts as a FAD-dependent oxidoreductase (EC 1.6.3.5), which oxidizes isomeric forms of β-NAD(P)H reduced at the 2 or 6 position of the nicotinamide ring instead of the metabolically active 4 position [5–7]. Extracellular RNLS, lacking the N-terminal signal peptide required for secretion of this protein into the extracellular space, exhibits numerous regulatory effects via non-catalytic mechanisms involving receptor proteins [8–11]. At the same time, our data indicate that intact RNLS is not detected in the blood [12], and recombinant RNLS in the blood plasma of healthy volunteers undergoes proteolytic processing [13]. This suggests that the effects of extracellular RNLS may be due to peptides generated during proteolysis of this protein. One of the most interesting renalase peptides that attracted the attention of researchers is the RP220 peptide (a 20-mer peptide corresponding to the amino acid sequence RNLS 220–239) [10, 11]. In addition to its effect on the survival of various cells [11, 14], there is evidence that RP220 increases the viability of human corneal epithelial cells after exposure to alkali [15], and also reduces the manifestations of lupus nephritis in MRL/lpr mice (a genetic model of systemic lupus erythematosus) [16].

Although some of these effects are associated with the involvement of a plasma membrane receptor (or receptors), certain evidence exists that RP220 and recombinant RNLS carry out rapid activation of intracellular protein kinases [10]. Taking into consideration these data, as well as “a piece of scant evidence” for the positive effect of recombinant RNLS in acute cell damage (see review [17]), it becomes important to analyze the potential intracellular targets of both RNLS itself and its peptides. In this context it should be noted that a recent proteomic analysis of HEK293T cells using recombinant proteins RNLS1 and RNLS2 as affinity ligands [18] showed their complete difference.

The aim of this study was to perform affinity based proteomic profiling of kidney tissue from normotensive Wystar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), derived from WKY, by using the renalase peptide RP220 as an affinity ligand.

MATERIALS AND METHODS

Reagents

The following reagents were used in this study: cyanogen bromide-activated Sepharose 4B, ammonium bicarbonate, dithiothreitol, urea, guanidine hydrochloride, 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 twenty-membered peptide RP220 with the amino acid sequence, corresponding to the human renalase fragment 220–239 (CIRFVSIDNKKRNIESSEIG), was synthesized by BelkiAntitela (Russia). The purity of this peptide was 98%. Other reagents of the highest purity available were from local suppliers.

Experimental Animals

Male 14-week-old WKY rats (n=6; blood pressure 110–120 mm Hg) and SHR (n=5; blood pressure 180 mm Hg or more) were used in experiments. The animals were obtained from the Nursery of the laboratory animals (Pushchino branch of the M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry RAS). 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) and diluted in it to a final concentration of 30 mg/ml. In order to evaluate relative quantitative changes in renal proteins the same amount of total protein was used during sample preparation; it was controlled by the Bradford method [8]. After incubation in the presence of 3% Triton X-100 (4°C, 1 h), the lysates were diluted 3 times with the same buffer and centrifuged at 16,000 *g* for 30 min to obtain a cleared supernatant.

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

The preparation of the affinity sorbent and the affinity chromatography procedure were carried as described in [20].

Identification and Comparison of the Relative Content of Proteins Pound to the Immobilized Renalase Peptide RP220 in WKY and SHR Rats

Sample preparation for mass spectrometric analysis (protein extraction, alkylation and trypsinolysis) was carried out as described previously [21]. Mass spectrometric analysis was carried out using the equipment of the "Human Proteome" Core Facility (Institute of Biomedical Chemistry). The details for mass spectrometric analysis and bioinformatics data processing are given in [22, 23].

RESULTS AND DISCUSSION

Proteomic analysis has shown significant changes in the relative content of a large number of kidney proteins bound to the renalase RP220 peptide in normotensive (WKY) and hypertensive (SHR) animals (Supplementary Material Tables S1 and S2, Tables 1 and 2, Figures 1 and 2). At the same time, we took into account the change in the relative content of proteins in the kidneys of SHR compared to normotensive rats [23]. For example, the relative content of superoxide dismutase [Cu-Zn] in the kidneys of hypertensive rats increased compared to that in control animals by less than 7 times, while the relative amount of this kidney enzyme bound to the RP220 peptide was 16-fold higher in SHR than in the case of the kidney enzyme of WKY rats. Similarly, for the lipoyl dehydrogenase component

Table 1. Rat kidney proteins (bound to the RP220 peptide) which demonstrate 9-fold or more pronounced increase in the relative content in hypertensive animals compared with the control animals

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	-LOG(P-value)	Fold increase	Role in cardiovascular pathology
1	O35331	*Pdxk*	Pyridoxal kinase	3	C	1.761	10.126	[24]
2	O70351	*Hsd17b10*	3-hydroxyacyl-CoA dehydrogenase type-2	7	Mch	4.770	51.732	[25, 26]
3	Q66HT1	*Aldob*	Fructose-bisphosphate aldolase B	1	C	2.640	45.664	[27, 28]
4	P02761	*Mup*	Major urinary protein	3	C, S	2.074	19.427	[29]
5	Q6LDS4	*Sod1*	Superoxide dismutase [Cu-Zn]	4	C, N	3.504	16.621	[30, 31]
6	P08461	*Dlat*	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	1	Mch	2.527	43.622	[32–34]
7	P09034	*Ass1*	Argininosuccinate synthase	6	C	4.856	12.193	[35]
8	P14408	*Fh*	Fumarate hydratase, mitochondrial	1	C, N	2.732	25.386	[36, 37]

Table 1. Rat kidney proteins (bound to the RP220 peptide) which demonstrate 9-fold or more pronounced increase in the relative content in hypertensive animals compared with the control animals (continue)

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	-LOG(P-value)	Fold increase	Role in cardiovascular pathology
9	P14604|	*Echs1*	Enoyl-CoA hydratase, mitochondrial	7	Mch	2.590	44.323	[38]
10	P19112	*Fbp1*	Fructose-1,6-bisphosphatase 1	1	C	4.084	159.786	[39]
11	P21533	*Rpl6*	60S ribosomal protein L6	5	C, ER	2.095	10.585	[40, 41]
12	P62718	*Rpl18a*	60S ribosomal protein L18a	5	C	2.184	9.815	[40, 41]
13	P98158	*Lrp2*	Low-density lipoprotein receptor-related protein 2	3	PM, ER, M	3.085	117.621	[42–44]
14	G3V6P2	*Dlst*	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	1	Mch	3.482	21.377	[33, 34, 45, 46]
15	G3V7J0	*Aldh6a1*	Aldehyde dehydrogenase 6 family, member A1	4	C	3.150	9.952	—
16	Q07523	*Hao2*	2-Hydroxyacid oxidase 2	7	Per	3.387	202.390	[47]
17	Q64057	*Aldh7a1*	Alpha-aminoadipic semialdehyde dehydrogenase	4	C, N, Mch	3.047	32.334	—
18	Q64428	*Hadha*	Trifunctional enzyme subunit alpha, mitochondrial	7	Mch	3.115	13.832	[48]
19	Q60587	*Hadhb*	Trifunctional enzyme subunit beta, mitochondrial	7	Mch	4.465	9.666	[49]
20	Q68FP1	*Gsn*	Gelsolin	2	C, S	2.631	9.279	[50, 51]
21	Q6AYS7	*Acy1a*	Aminoacylase-1A	6	C	4.640	208.225	[52, 53]
22	Q6AYT0	*Cryz*	Quinone oxidoreductase	1	C	2.930	29.712	[54, 55]
23	Q6IRK9	*Cpq*	Carboxypeptidase Q	6	ER, G, L	3.608	43.865	—
24	Q7M0E	*Dstn*	Destrin	2	C	3.047	32.334	[56, 57]
25	Q7TPB1	*Cct4*	T-complex protein 1 subunit delta	4	C	3.115	13.832	[58]
26	Q63716	*Prdx1*	Peroxiredoxin-1	4	C	6.248	10.140	[59–62]
27	P35704	*Prdx2*	Peroxiredoxin-2	4	C	2.649	8.907	[59, 63]
28	A0A0G2JSS8	*Prdx5*	Peroxiredoxin-5	4	C	4.640	208.225	[59]
29	Q9WUW9	*Sult1c2a*	Sulfotransferase 1C2A	3	C, L	2.930	29.712	[60]
30	F1LMC7	*Septin7*	Septin 7	3	C, N	3.608	43.865	[64]
31	F7FKI5	*Pdha1*	Pyruvate dehydrogenase E1 component subunit alpha	1	Mch	3.738	21.288	[32, 65]
32	A6JVU7	*Vil1*	Vil1 protein	2	C	4.858	144.407	[66]
33	D4A830	*Ppa2*	Inorganic diphosphatase	3	C, Mch	6.498	141.828	[67, 68]
34	F1LR02	*Col18a1*	Collagen type XVIII alpha 1 chain	2	PM	6.010	9.917	[69]
35	G3V8T4	*Ddb1*	DNA damage-binding protein 1	5	N	2.020	16.111	[70, 71]

Here and in Table 2, the numbers in the column "Function" indicate the following functional groups of proteins: *1.* Proteins/enzymes involved in the processes of energy generation and carbohydrate metabolism. *2.* Proteins involved in the formation of the cytoskeleton, transport and exocytosis. *3.* Proteins involved in signal transmission and regulation of enzyme activity. *4.* Antioxidant and protective proteins/enzymes. *5.* Proteins that regulate 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. Localization of proteins: C – cytoplasm, N – nucleus, M – membranes, PM – plasma membrane, Mch – mitochondria, Mi – microsomes, Me – melanosomes, ER – endoplasmic reticulum, G – Golgi complex, L – lysosomes, Ve – vesicles, Per – peroxisomes, S – secreted proteins.

Table 2. Rat kidney proteins (bound to the RP220 peptide) which demonstrate 4-fold or more pronounced decrease in the relative content in hypertensive animals compared with the control animals

#	Uniprot accession number	Uniprot gene name	Uniprot protein name	Function	Localization	-LOG(P-value)	Fold decrease	Role in cardiovascular pathology
1	P05197	*Eef2*	Elongation factor 2	5	C, N	4.039	0.239	[72]
2	P07150	*Anxa1*	Annexin A1	2	PM, C	1.907	0.237	[73]
3	P17078	*Rpl35*	60S ribosomal protein L35	5	C	1.313	0.219	[40, 41]
4	P19945	*Rplp0*	60S acidic ribosomal protein P0	5	C, N	2.273	0.091	[40. 41]
5	P20280	*Rpl21*	60S ribosomal protein L21	5	C, ER	1.188	0.245	[40, 41]
6	P38983|	*Rpsa*	40S ribosomal protein SA	5	PM, M, N, C	2.343	0.154	[40, 41]
7	P40112	*Psmb3*	Proteasome subunit beta type-3	6	C, N	2.171	0.113	[74–76]
8	P48679	*Lmna*	Prelamin-A/C	2	N	2.922	0.190	[77]
9	P63174	*Rpl38*	60S ribosomal protein L38	5	C	1.452	0.233	[40, 41]
10	P85108	*Tubb2a*	Tubulin beta-2A chain	2	C, Mch	2.128	0.124	[78]
11	Q4FZT9	*Psmd2*	26S proteasome non-ATPase regulatory subunit 2	6	C, N	1.788	0.032	[74–76]
12	Q5U216	*Ddx39a*	ATP-dependent RNA helicase DDX39A	5	C, N	1.354	0.071	[79]
13	A0A0H2UHM7	*Tuba1c*	Tubulin alpha chain	2	C	3.962	0.086	[78]
14	F1M5A4	*Katnal2*	Katanin p60 ATPase-containing subunit A-like 2	2	C	2.449	0.219	[78]
15	P48004	*Psma7*	Proteasome subunit alpha type-7	6	C, N	3.111	0.226	[74–76]
16	G3V9G4	*Acly*	ATP-citrate synthase	1	C	2.156	0.158	[80]
17	P52296|	*Kpnb1*	Importin subunit beta-1	3	C, N	3.573	0.128	—
18	G3V7C6	*Tubb4b*	Tubulin beta chain	2	C	4.010	0.248	[78]
19	Q6AYD5	*Gspt1*	G1 to S phase transition protein 1	5	N, C	2.179	0.164	—
20	Q6P3V8	*Eif4a1*	ATP-dependent RNA helicase	5	C	3.053	0.239	[79]

Table 3. Functional distribution of renal proteins (bound to the RP220 peptide) with significantly altered relative content in hypertensive rats

Functional group	Increased relative content	Decreased relative content
Proteins/enzymes involved in the processes of energy generation and carbohydrate metabolism	7	1
Proteins involved in the formation of the cytoskeleton, transport and exocytosis	4	6
Proteins involved in signal transduction and regulation of enzyme activity	6	1
Antioxidant and protective proteins/enzymes	7	—
Proteins involved in regulation of gene expression, cell division, and differentiation	3	9
Enzymes involved in metabolism of proteins, amino acids and other nitrogenous compounds	3	3
Enzymes involved in lipid metabolism	5	—
Total	35	20

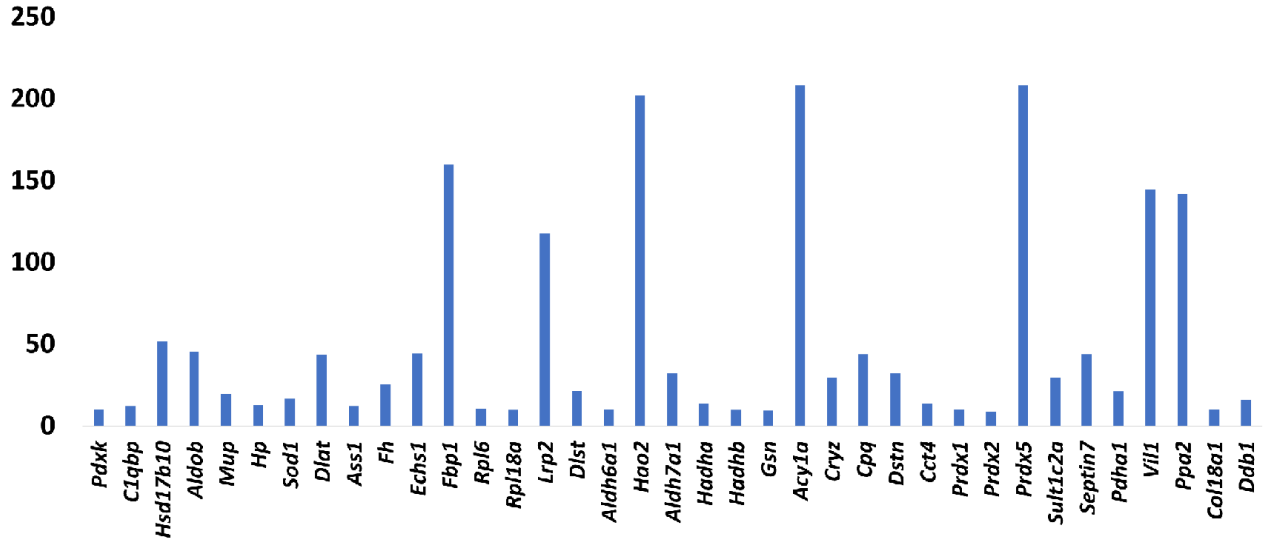

Figure 1. The most pronounced increase in the relative content of renal RP220-bound proteins in hypertensive rats as compared to that of control normotensive animals (indicated using the gene names of corresponding protein products).

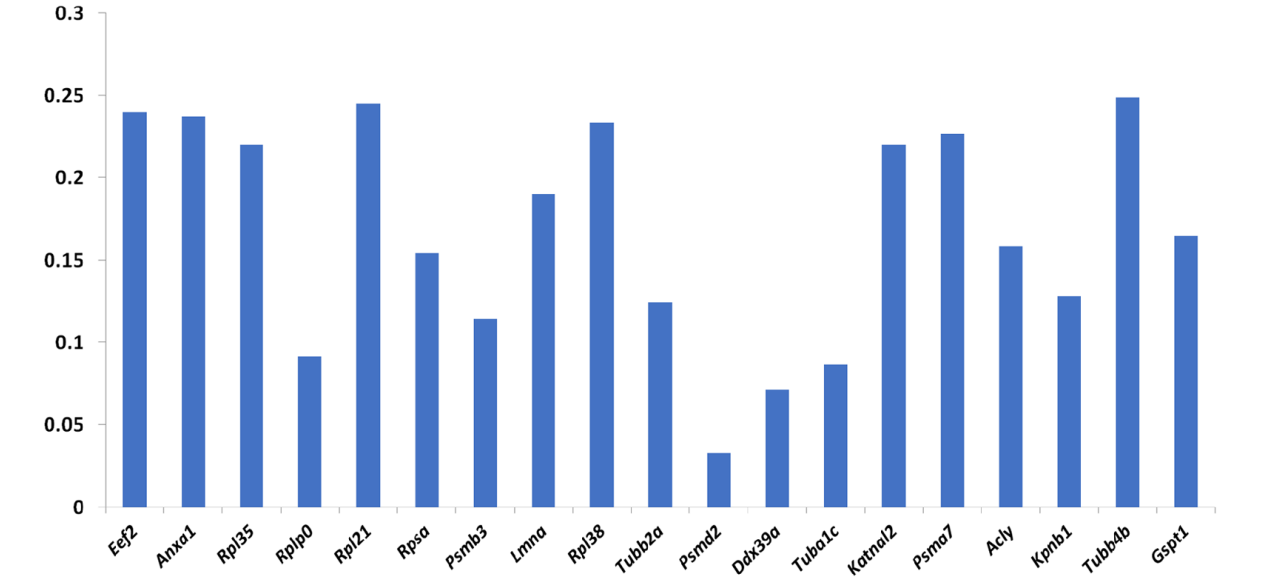

Figure 2. The most pronounced decrease in the relative content of renal RP220-bound proteins in hypertensive rats as compared to that of control normotensive animals (indicated using the gene names of corresponding protein products).

of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes (the increase in the relative content of this enzyme in the kidneys of SHR rats in both cases was 3 times compared to the WKY rats, while in the case of its binding to the RP220 peptide, the increase was more than 43 times and more than 21 times, respectively). The relative content of sulfotransferase 1C2A bound to the RP220 peptide was 30 times higher in SHR as compared with control normotensive animals [23], while the relative content of this protein was 2-times lower in SHR as compared to WKY rats.

In hypertensive rats, the relative content of 98 kidney proteins bound to RP220 was higher and the relative content of 90 proteins bound to RP220 was lower than in corresponding kidney proteins of WKY rats bound to this affinity sorbent (see Supplementary Table S1 and S2). Tables 1 and 2 and also Figures 1 and 2 show proteins demonstrating the most pronounced changes in SHR compared to WKY rats.

An increase in the relative content by 9 or more times was shown for 35 proteins. Almost all of them are associated with hypertension and/or cardiovascular pathology (Table 1). Table 3 shows the functional distribution of proteins bound to the RP220 peptide. Seven enzymes belong to the group of proteins involved in the processes of energy generation and carbohydrate metabolism. These are the glycolytic enzyme fructose bisphosphate aldolase B [27, 28], Krebs cycle enzymes: fumarate hydratase [36, 37], dihydrolipoyl dehydrogenase component of pyruvate dehydrogenase [32–34] and 2-oxoglutarate dehydrogenase mitochondrial enzyme complexes [33, 34, 45, 46],

alpha subunit E1 of the pyruvate dehydrogenase complex component [32, 65], — as well as the multienzyme complex of the respiratory chain NADH-ubiquinone oxidoreductase [54, 55] and the key enzyme of gluconeogenesis, fructose-1,6-bisphosphatase 1 [39].

Seven RP220 binding proteins demonstrating the most pronounced relative increase in SHR belong to the functional group of protective and antioxidant proteins. These include peroxiredoxins 1, 2, and 5 [59–63], a component of the chaperonin-containing T-complex that regulates protein folding, superoxide dismutase [Cu-Zn] [30, 31], aldehyde dehydrogenase, and the multifunctional enzyme alpha-aminoadipate semialdehyde dehydrogenase.

Six SHR proteins characterized by increased relative binding to the RP220 peptide (versus WKY rats) belong to the functional group of proteins involved in signal transmission and regulation of enzyme activity. These include pyridoxal kinase [24], a major urinary protein [29], low-density lipoprotein receptor family protein [42–44], sulfotransferase 1C2A [60], septin 7 [64], and inorganic diphosphatase [67, 68].

A significant increase in the relative content of SHR kidney proteins bound to the RP220 peptide was also found for five enzymes of lipid metabolism: 3-hydroxyacyl-CoA dehydrogenase type 2 [25, 26], mitochondrial enoyl CoA hydratase [38], peroxisomal glycolate oxidase [47], alpha and beta subunits of the mitochondrial trifunctional enzyme [48, 49].

The most pronounced relative content of SHR kidney proteins bound to the RP220 was also found for four proteins of cytoskeleton, transport and exocytosis, three proteins involved in regulation of gene expression, cell division and differentiation, and three enzymes of metabolism of proteins, amino acids, and other nitrogenous compounds (Tables 1 and 3).

As can be seen from the literature data given in Table 1, all of these proteins are associated with cardiovascular pathology. For example, proteomic studies performed using salt-sensitive (SS) rats (an animal model of hypertension) revealed a significant increase in the content of Krebs cycle metabolites and glycolysis in these animals and a corresponding decrease in the activity of enzymes involved in these processes [45].

Convincing evidence exists that oxidative stress plays an important role in the development of cardiovascular pathology. Antioxidant enzymes, such as superoxide dismutases (SOD), peroxiredoxins and others, maintain the balance of reactive oxygen species in the body by preventing their sharp increase determining the development of cardiovascular disorders. It was shown that the activity of Cu/Zn SOD (a scavenger of reactive oxygen species) in SHR rats kept on a zinc-depleted diet was significantly lower than in the animals kept on a standard diet and correlated with high blood pressure [30, 31]. Peroxiredoxins 1 and 2 play a significant role in the prevention of atherosclerosis, myocarditis, and thrombus formation, and therefore these proteins are considered as targets for therapeutic strategies in the treatment of cardiovascular diseases [59–63]. The role of peroxiredoxin 5 in the pathogenesis of these disorders is less studied [59]; however, our results indicate that the increase in the relative content of this protein bound to the RP220 peptide in the kidneys of SHR rats, compared with control normotensive (WKY) animals, is an order of magnitude higher than the increase in the content of peroxiredoxin 1 and 2 (Table 1, Fig. 1).

Among the SHR kidney regulatory proteins, the most significant increase in the relative content of proteins bound to the RP220 peptide (versus corresponding WKY proteins) was observed in a protein belonging to the low-density lipoprotein receptor family and in inorganic diphosphatase. Members of the low-density lipoprotein receptor family are extensively studied in the context of atherosclerosis, kidney disease, and pulmonary hypertension. These proteins are involved not only in the regulation of lipid metabolism, but also in many cellular processes, interacting with various ligands and receptors [42–44]. Regarding inorganic diphosphatase, the role of phosphate in the development of hypertension is widely known [68]. At the same time, it is known that mutations in the mitochondrial inorganic diphosphatase encoded by nuclear DNA are associated with cardiomyopathy leading to early death [67].

The increased relative content of glycolate oxidase (L-2-hydroxy acid oxidase), the enzyme involved in lipid metabolism and bound to the RP220 peptide, represented one of the highest differences of SHR from WKY rats. This peroxisomal enzyme is predominantly expressed in the liver and kidney. The L-2-hydroxy acid oxidase gene was recently identified as a quantitative trait locus for blood pressure. Selective inhibitors of this enzyme are being studied in animal models of hypertension with the aim of developing appropriate therapeutic strategies [47].

Besides renal SHR proteins demonstrating increased relative binding to the RP220 peptide (versus WKY renal proteins), there were renal SHR proteins demonstrating decreased relative binding to the RP220 peptide as compared to renal WKY proteins. They were from functional groups of protein synthesis and degradation and also from cytoskeletal components (Table 2, Fig. 2). These included ribosomal and proteasomal subunits, elongation factor eEF2, components of microtubules and associated proteins.

For certain rodent and human ribosomal proteins a link between their altered expression and the regulation of the growth of smooth muscle cells and, as a consequence, with cardiovascular pathology has been recognized [40, 41]. Elongation factor eEF2, a protein that determines the ribosome movement along mRNA from one codon to another during translation,

is also associated with cardiovascular disorders. Elongation factor phosphorylating protein kinase, which is inhibited by its own substrate and thus regulates its activity, is known to be associated with atherosclerosis and pulmonary hypertension [72].

The ubiquitin-proteasome system (UPS) maintains the dynamic balance of proteins in the cell, controlling many biological processes and interacting with a number of proteins that form proteasomal proteomes [81–84]. Many studies on UPS are aimed at the regulation of proteasome function in the context of oxidative stress [85–87]. The UPS dysfunction has been shown in cardiovascular diseases: hypertrophic cardiomyopathy [74], pulmonary hypertension [75]. Proteasome regulators are considered as pharmacotherapy agents of cardiovascular disorders [76]. It is therefore not surprising that the list of renal SHR proteins demonstrating decreased relative binding to the RP220 peptide (versus WKY control) contained catalytic and regulatory proteasome subparticles (Table 2, Fig. 2).

Another group of renal SHR proteins demonstrating decreased relative binding to the RP220 peptide (versus WKY control) included microtubule proteins and microtubule associated proteins: alpha and beta chains of tubulin, annexin, katanin, prelamin (Table 2, Fig. 2). Impaired functioning of microtubules and cytoskeletal components interacting with them plays certain role in the development of cardiovascular pathology [73, 76, 78].

CONCLUSIONS

The results obtained in this study indicate that, in addition to intracellular protein kinases that interact with RP220 [10], kidney cells contain many proteins that may represent potential targets for this peptide. The significance of this interaction remains unclear and requires further research. However, altered interactions (in the context of hypertension-normotension) of proteins of certain functional groups, especially proteins, involved in the development of cardiovascular pathology, indicate the potential biomedical importance of the discovered changes.

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COMPLIANCE WITH ETHICAL STANDARDS

The experiments were carried out in compliance with generally accepted norms of humane treatment of laboratory animals. The work was carried out 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” and the Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010 on the protection of animals used for scientific purposes.

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).

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ПРОТЕОМНОЕ ПРОФИЛИРОВАНИЕ ПОЧЕК НОРМО- И ГИПЕРТЕНЗИВНЫХ КРЫС С ИСПОЛЬЗОВАНИЕМ РЕНАЛАЗНОГО ПЕПТИДА RP220 В КАЧЕСТВЕ АФФИННОГО ЛИГАНДА

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Реналаза (RNLS) — недавно открытый белок, который играет важную роль в регуляции артериального давления, действуя внутри и снаружи клеток. Внутриклеточная RNLS — FAD-зависимая оксидоредуктаза, которая осуществляет окисление изомерных форм β-NAD(P)H. Внеклеточная реналаза, лишённая своего N-концевого пептида и кофактора FAD, проявляет различные защитные эффекты при помощи некаталитических механизмов. По данным ряда авторов, пептид RP220 (20-членный пептид, соответствующий аминокислотной последовательности RNLS 220–239) воспроизводит ряд некаталитических эффектов этого белка, действуя на рецепторные белки плазматической мембраны. Возможность взаимодействия этого пептида с внутриклеточными белками не изучена. С учётом известной роли RNLS как возможного антигипертензивного фактора, в данной работе осуществлено протеомное профилирование почек нормо- и гипертензивных крыс с использованием RP220 в качестве аффинного лиганда. Протеомная (полуколичественная) идентификация выявила изменения относительного содержания связавшихся с аффинным сорбентом около 200 индивидуальных белков почек гипертензивных крыс по сравнению с почками нормотензивных животных. При этом оказалось, что связавшиеся с RP220 белки, для которых обнаружено наиболее выраженное увеличение относительного содержания у гипертензивных животных по сравнению с нормотензивными, вовлечены в развитие сердечно-сосудистой патологии. Снижение связывания с RP220 белков почек гипертензивных животных отмечено для компонентов убиквитин-протеасомной системы, рибосом и цитоскелета.

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

Ключевые слова: реналаза; пептиды реналазы; артериальная гипертензия; крысы WKY и SHR; протеомное профилирование ткани почек

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