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CHANGES IN THE EXPRESSION OF MOLECULAR GENETIC REGULATORS OF THE FUNCTIONAL STATE OF ENDOCRINE CELLS IN SHR RATS

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Abstract

Essential arterial hypertension is accompanied by chronic vascular and neurohumoral stress and microcirculatory disturbances that can alter the functional state of the endocrine compartment of the pancreas and promote its remodelling. The molecular mechanisms underlying these changes under conditions of hereditary hypertension remain insufficiently defined, particularly with respect to the involvement of vasoactive, inflammatory, and hypoxia-associated gene networks. In this context, SHR rats represent a relevant model of hereditary hypertension for a systematic assessment of expression changes in key hypertension-related genes in pancreatic tissue.

The aim of the work: to determine changes in the expression of key molecular and genetic regulators that characterise the functional state of pancreatic endocrine cells in SHR rats under conditions of hereditary hypertension.

Materials and methods. For the analysis of gene expression, the real-time reverse transcription polymerase chain reaction method was used using the PARN-037Z RT² Profiler™ PCR Array Rat Hypertension (QIAGEN, Germany), where the pancreas was the object of the study in experimental animals.

Results. In SHR rats, pancreatic tissue showed a coordinated increase in the expression of neurohumoral and vasoactive genes, including *Ren* (8.87-fold), *Agtr2* (4.96-fold), *Ece1* (3.42-fold), *Ednra* (4.65-fold), and *Avpr1b* (8.10-fold) ($2^{-}\Delta\Delta Ct$), indicating activation of local renin-angiotensin, endothelin, and vasopressin-related pathways. A hypoxia-associated response was evident, with *Hif1a* upregulated 5.37-fold. Marked dysregulation of ion handling and calcium signalling was observed (*Kcnj8* 2.08-fold, *Kcnma1* 5.16-fold, *Cngb1* 5.30-fold, *Itpr3* 3.59-fold, *P2rx4* 5.51-fold), accompanied by activation of cytoskeletal and contractile regulators (*Mylk* 3.01-fold, *Mylk2* 6.69-fold). Stress-related neuropeptide systems were strongly induced (*Calca* 6.34-fold, *Nppb* 196.15-fold), while arachidonic acid-related inflammatory and vasoactive pathways increased (*Ptgs1* 2.34-fold, *Ephx2* 8.80-fold) together with metabolic transport changes (*Slc7a1* 15.47-fold) ($2^{-}\Delta\Delta Ct$). Collectively, these shifts support a pattern of chronic neurohumoral overload, hypoxia, ionic and metabolic dysregulation in the pancreas under hereditary hypertension.

Conclusions: 1. In SHR rats, increased expression of *Ren*, *Agtr2*, *Ece1*, *Ednra*, and *Avpr1b* indicates pathological activation of local neurohumoral systems (renin-angiotensin, endothelin, and vasopressin signalling), which is associated with vasoconstriction, impaired microcirculation, and reduced perfusion of the islets of Langerhans.

2. Upregulation of *Hif1a* indicates the development of chronic tissue hypoxia in the pancreas under long-term hypertension, creating an unfavourable microenvironment and potentially reducing glucose-stimulated insulin secretion.

3. Increased expression of ion channel and calcium signalling genes (*Kcnj8, Kcnma1, Cngb1, Itpr3, P2rx4*), together with cytoskeletal and contractile regulators (*Mylk, Mylk2*), reflects disorganisation of ion homeostasis and exocytosis, which may contribute to calcium overload and endocrine cell dysfunction.

4. Activation of stress-related and pro-inflammatory, vasoactive pathways (*Calca, Nppb, Ptgs1, Ephx2, Slc7a1*) is consistent with metabolic overload and inflammatory, oxidative, and nitrosative stress, thereby amplifying the damaging effects of chronic arterial hypertension and reducing the reserve capacity of the endocrine compartment.

Keywords: SHR rats; pancreas; genes; essential arterial hypertension; endocrine cells.

Introduction. Essential arterial hypertension is a systemic chronic condition that extends beyond an isolated elevation of arterial pressure and is accompanied by microcirculatory disturbances, endothelial dysfunction, oxidative stress, and neurohumoral activation. These changes create a cardiometabolic context in which arterial hypertension is often combined with insulin resistance and an increased risk of disorders of carbohydrate metabolism [1]. Accordingly, the pancreas-particularly its endocrine compartment-represents a potential target of prolonged vascular and metabolic load.

The endocrine compartment of the pancreas is a dynamic system capable of responding to chronic stressors through structural and functional remodelling. A key concept that explains the direction and consequences of such rearrangements is cellular plasticity: differentiated cells can modify transcriptional programs, functional properties, and features of cellular identity under the influence of the microenvironment and damaging factors [2]. In contemporary views on endocrine pathology, processes of loss of specialised characteristics (dedifferentiation) and reprogramming may play an important role and can determine endocrine cell dysfunction alongside cell death [3].

The SHR model is among the most widely used experimental approaches for studying mechanisms of essential arterial hypertension and associated metabolic shifts [4]. In this model, arterial hypertension has been shown to be associated with changes in the islet apparatus, including enlargement of islets and increased cellular replication accompanied by reduced proinsulin and insulin biosynthesis and alterations in islet blood flow [5]. The microcirculatory component is of particular importance: in SHR, impaired islet microvascular vasomotion has been described, with a loss of the ability to adequately regulate perfusion and a reduction in perfusion patterns [6], and changes in pancreatic microcirculation during the progression of hypertension have also been demonstrated [7]. These data support the assumption that the microenvironment (perfusion, vascular tone, local mediator circuits) may be a key modifier of the transcriptional state of endocrine cells and the direction of remodelling.

An additional link connecting vascular and endocrine effects is the local islet renin-angiotensin system, its components can influence endocrine cell function, inflammatory responses, and microvascular tone, thereby modulating the conditions under which the endocrine compartment operates [8]. Taken together, the combination of microcirculatory disorders, neurohumoral activation, and potential epigenetic and transcriptional reprogramming provides a rationale for a systematic assessment of molecular and genetic

determinants that characterise the functional state of pancreatic endocrine cells under conditions of hereditary hypertension.

Therefore, it is relevant to determine changes in the expression of key genes associated with cellular identity, plasticity, energy resilience, and interactions with the microenvironment in pancreatic tissue of SHR rats. This approach makes it possible to link morphofunctional manifestations of remodelling with molecular mechanisms that may underlie endocrine dysfunction under chronic hypertensive stress.

The aim of the work: to determine changes in the expression of key molecular and genetic regulators that characterise the functional state of pancreatic endocrine cells in SHR rats under conditions of hereditary hypertension.

Materials and methods of the study. The study was conducted on 10 white rats, which were divided into two groups (5 animals in each group). Animals in Group 1 constituted the control group (normotensive Wistar rats). Animals in Group 2 constituted the experimental group-SHR rats (spontaneously hypertensive rats), which served as a model of essential arterial hypertension.

After decapitation of the experimental animals under thiopental anaesthesia (50 mg/kg), the pancreas was collected, fixed in Buena's solution (20 hours), and, after standard histological processing, embedded in Paraplast (McCormick, USA).

Gene expression was analysed using real-time reverse transcription polymerase chain reaction with the PARN-037Z RT² Profiler™ PCR Array Rat Hypertension (QIAGEN, Germany), with the pancreas serving as the study object in the experimental animals. Polymerase chain reaction data were analysed using PCR GeneGlobe software (QIAGEN, Germany) with the $2^{-}\Delta\Delta Ct$ method [9].

Results. The PARN-037Z RT² Profiler™ PCR Array Rat Hypertension gene panel (QIAGEN), which comprises 84 hypertension-related genes involved in vasoactive and neurohumoral regulation, vascular function, inflammation, oxidative stress, extracellular matrix remodelling, and hypoxia-associated responses, was used to provide a molecular characterisation of pancreatic changes under conditions of hereditary arterial hypertension in SHR rats. The obtained results reflect gene expression changes in pancreatic tissue as a whole and therefore describe key molecular pathways through which hypertensive stress may modulate the functional state of pancreatic endocrine cells and their microenvironment.

Based on the gene expression analysis in pancreatic samples from control animals and hypertensive SHR rats, the activity of the genes included in the polymerase chain reaction panel was systematised according to the direction of expression changes as follows: genes

with increased expression compared with the control group; genes with decreased expression compared with the control group; genes with no detectable changes in expression relative to the control group; and genes whose expression was not detected (Table 1).

Table 1 - Gene expression profile of the PARN-037Z RT² Profiler™ PCR Array Rat Hypertension panel in SHR rats compared with the control group ($2^{-}\Delta\Delta$Ct method)

	1	2	3	4	5	6	7	8	9	10	11	12
A	*Ace*	*Ace2*	*Acta2*	*Adm*	*Adra1b*	*Adra1d*	*Adrb1*	*Agt*	*Agtr1a*	*Agtr1b*	*Agtr2*	*Alox5*
B	*Arg2*	*Atp2c1*	*Atp6ap2*	*Avp*	*Avpr1a*	*Avpr1b*	*Bdkrb1*	*Bdkrb2*	*Bmpr2*	*Cacna1c*	*Calca*	*Cav1*
C	*Chrna1*	*Chrnb1*	*Clic1*	*Clic4*	*Clic5*	*Cnga1*	*Cnga2*	*Cnga3*	*Cnga4*	*Cngb1*	*Cps1*	*Drd3*
D	*Drd5*	*Ece1*	*Edn1*	*Edn2*	*Ednra*	*Ednrb*	*Ephx2*	*Gch1*	*Gchfr*	*Gucy1a1*	*Gucy1b1*	*Hif1a*
E	*Itpr1*	*Itpr2*	*Itpr3*	*Kcnj8*	*Kcnma1*	*Mylk*	*Mylk2*	*Mylk3*	*Nos3*	*Nosip*	*Nostrin*	*Nppb*
F	*Nppc*	*Npr1*	*Npy1r*	*P2rx4*	*Pde3a*	*Pde3b*	*Pde5a*	*Plcg1*	*Plcg2*	*Prkg1*	*Prkg2*	*Ptgir*
G	*Ptgs1*	*Ptgs2*	*Ren*	*S1pr1*	*Scnn1a*	*Scnn1b*	*Scnn1g*	*Slc7a1*	*Sphk1*	*Sphk2*	*Uts2*	*Uts2r*

Notes: 1. Green indicates genes with increased expression. Red indicates genes with decreased expression. Yellow indicates genes with no changes in expression (≈1).Gray indicates genes with undetected expression (for the listed genes, expression was not detected either in the control group or in the group of animals under EDM conditions. The threshold cycle values were at the sensitivity limit of the RT-qPCR method (Ct ≈ 40), which indicates the absence of detected transcriptional activity in the experimental conditions studied).

2. Data are presented as average values (AVG) normalized to reference genes according to the PCR Array algorithm.

Discussion. In this article, we focus on the characteristics of messenger ribonucleic acid expression of genes that demonstrated increased expression activity (Table 2).

The detected increase in the expression of a number of genes (Table 2) in the pancreas of rats with hereditary arterial hypertension indicates a set of changes directed not only toward reconfiguration of regulatory mechanisms, but also toward the development of chronic stress and injury of the endocrine compartment of the pancreas under conditions of long-term arterial hypertension [10].

An increase in *Ren* by 8.87-fold, *Agtr2* by 4.96-fold, *Ece1* by 3.42-fold, *Ednra* by 4.65-fold, and *Avpr1b* by 8.10-fold ($2^{-}\Delta\Delta$Ct method) in pancreatic tissue indicates pathological activation of local neurohumoral systems.

Table 2 - Genes with increased expression relative to the control group, based on analysis using the $2^{-}\Delta\Delta Ct$ method.

Hole	Gene	Average Amplification Cycle Threshold (Avg Ct) Control	Average Amplification Cycle Threshold (Avg Ct) SHR	The mean value is the test gene/reference gene (ΔCt). Control	The mean value is the test gene/reference gene (ΔCt). SHR	Normalized relative expression level of the studied gene ($2^{-}\Delta Ct$). Control	Normalized relative expression level of the studied gene ($2^{-}\Delta Ct$). SHR	Fold change in expression
A11	*Agtr2*	39.140	40.000	3.160	0.860	0.112	0.553	4.96
B06	*Avpr1b*	39.850	40.000	3.870	0.860	0.068	0.553	8.10
B11	*Calca*	34.720	35.230	-1.250	-3.920	2.381	15.107	6.34
C05	*Clic5*	38.870	38.080	2.890	-1.060	0.135	2.091	15.49
C10	*Cngb1*	38.360	39.130	2.390	-0.020	0.191	1.013	5.30
C11	*Cps1*	40.000	39.940	4.020	0.800	0.061	0.576	9.37
D02	*Ece1*	38.610	40.000	2.630	0.860	0.162	0.553	3.42
D05	*Ednra*	39.050	40.000	3.070	0.860	0.119	0.553	4.65
D07	*Ephx2*	37.040	37.070	1.060	-2.070	0.479	4.212	8.80
D12	*Hif1a*	39.260	40.000	3.280	0.860	0.103	0.553	5.37
E03	*Itpr3*	38.680	40.000	2.700	0.860	0.154	0.553	3.59
E04	*Kcnj8*	37.880	40.000	1.910	0.860	0.266	0.553	2.08
E05	*Kcnma1*	39.200	40.000	3.220	0.860	0.107	0.553	5.16
E06	*Mylk*	38.420	40.000	2.440	0.860	0.184	0.553	3.01
E07	*Mylk2*	39.570	40.000	3.600	0.860	0.083	0.553	6.69
E12	*Nppb*	40.000	35.550	4.020	-3.590	0.061	12.057	196.15
F04	*P2rx4*	34.890	35.600	-1.090	-3.550	2.122	11.694	5.51
G01	*Ptgs1*	38.060	40.000	2.080	0.860	0.236	0.553	2.34
G03	*Ren*	39.980	40.000	4.000	0.860	0.062	0.553	8.87
G08	*Slc7a1*	34.540	33.760	-1.430	-5.390	2.702	41.796	15.47

Note: Data are presented as average values (AVG) normalized to reference genes according to the PCR Array algorithm.

Excessive activity of the local renin-angiotensin and endothelin systems in the pancreas is associated with vasoconstriction, reduced perfusion of the islets of Langerhans, and impaired microcirculation [11]. Activation of *Ren* and angiotensin receptors under hypertensive conditions may promote the development of ischaemic changes in pancreatic tissue, negatively affecting the functional state of endocrine cells. Increased expression of *Ece1* and *Ednra* additionally enhances the vasoconstrictor effects of endothelin-1, which is considered one of the key factors driving microvascular injury and endothelial dysfunction [12].

Increased *Avpr1b* expression reflects engagement of stress-implementing neuroendocrine mechanisms, which, under prolonged exposure, may lead to exhaustion of secretory activity in islet cells [13].

Elevated *Hif1a* expression by 5.37-fold ($2^{-}\Delta\Delta$Ct method) indicates the development of chronic tissue hypoxia in the pancreas of rats with hereditary arterial hypertension. Long-term hypertension is accompanied by vascular remodelling, reduced capillary density, and impaired oxygen delivery, creating an unfavourable microenvironment for endocrine cells [14]. Sustained activation of *Hif1a* in beta cells is regarded as a factor that disrupts aerobic metabolism, reduces glucose-stimulated insulin secretion, and promotes cellular dysfunction [15].

Increased expression of *Kcnj8* by 2.08-fold, *Kcnma1* by 5.16-fold, *Cngb1* by 5.30-fold, *Itpr3* by 3.59-fold, and *P2rx4* by 5.51-fold ($2^{-}\Delta\Delta$Ct method) reflects substantial disorganisation of endocrine cell ion homeostasis. Excessive activation of ion channels and calcium signalling pathways can lead to membrane potential instability and pathological intracellular calcium overload [16]. Chronic elevation of intracellular calcium is a well-established mechanism of beta-cell injury, triggering endoplasmic reticulum stress, impaired exocytosis, and initiation of apoptosis [16].

An increase in *Mylk* expression by 3.01-fold and *Mylk2* by 6.69-fold ($2^{-}\Delta\Delta$Ct method) indicates activation of contractile and cytoskeletal mechanisms that, under chronic load, may lose regulated control. Imbalance of the actin–myosin system adversely affects insulin granule trafficking and results in inefficient or disorganised exocytosis [17].

Elevated expression of *Calca* by 6.34-fold and *Nppb* by 196.15-fold ($2^{-}\Delta\Delta$Ct method) reflects activation of stress-implementing neuropeptide systems. Excessive expression of these genes in tissues is associated with impaired intercellular communication and changes in local vascular tone, which may deepen ischaemic processes [18].

Increased *Ptgs1* expression by 2.34-fold and *Ephx2* by 8.80-fold ($2^{-}\Delta\Delta$Ct method) indicates activation of pro-inflammatory and vasoactive arachidonic acid metabolites. Persistent upregulation of these pathways is linked to chronic inflammation, oxidative stress, and reduced beta-cell glucose sensitivity [19].

Elevated *Slc7a1* expression by 15.47-fold ($2^{-}\Delta\Delta$Ct method) suggests disturbances in amino acid metabolism and metabolic overload. Excessive arginine transport may contribute to imbalance of nitric oxide-dependent mechanisms, which under hypertensive conditions is associated with nitrosative stress and additional endocrine cell injury [20].

The obtained results indicate that increased expression of *Agtr2, Avpr1b, Calca, Clic5, Cngb1, Cps1, Ece1, Ednra, Ephx2, Hif1a, Itpr3, Kcnj8, Kcnma1, Mylk, Mylk2, Nppb, P2rx4, Ptgs1, Ren*, and *Slc7a1* in pancreatic tissue of rats with hereditary arterial hypertension is a molecular reflection of the damaging impact of chronic arterial hypertension. These changes characterise a state of chronic hypoxia, neurohumoral overload, and ionic and metabolic dysregulation, thereby creating prerequisites for progressive endocrine cell dysfunction and an overall reduction in reserve capacity of the endocrine compartment.

Conclusions: 1. In SHR rats, increased expression of *Ren*, *Agtr2*, *Ece1*, *Ednra*, and *Avpr1b* indicates pathological activation of local neurohumoral systems (renin-angiotensin, endothelin, and vasopressin signalling), which is associated with vasoconstriction, impaired microcirculation, and reduced perfusion of the islets of Langerhans.

2. Upregulation of *Hif1a* indicates the development of chronic tissue hypoxia in the pancreas under long-term hypertension, creating an unfavourable microenvironment and potentially reducing glucose-stimulated insulin secretion.

3. Increased expression of ion channel and calcium signalling genes (*Kcnj8, Kcnma1, Cngb1, Itpr3, P2rx4*), together with cytoskeletal and contractile regulators (*Mylk, Mylk2*), reflects disorganisation of ion homeostasis and exocytosis, which may contribute to calcium overload and endocrine cell dysfunction.

4. Activation of stress-related and pro-inflammatory, vasoactive pathways (*Calca, Nppb, Ptgs1, Ephx2, Slc7a1*) is consistent with metabolic overload and inflammatory, oxidative, and nitrosative stress, thereby amplifying the damaging effects of chronic arterial hypertension and reducing the reserve capacity of the endocrine compartment.

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