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The features of beta-cells organization in the pancreas of spontaneously hypertensive rat (SHR)

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Key words: *β-cells, Insulin, Hypertension.*

The control of β-cells pool in patients with hypertension is an actual problem, as it is possible that the hereditary genetic defects of arterial hypertension formation may affect the mechanisms of endocrine cell mass maintenance in pancreas and cause disruption of glucose metabolism and diabetes. In addition, violation of cytoarchitectonics of pancreatic islets may affect the adequate insulin secretion by the pancreas. This could be provoked by regional blood flow violations in patients with hypertension.

The aim of our study was to assess the parameters of the allocation of pancreatic islets and characterize the β-cells morphofunctional state in SHR.

Materials and methods. We assessed the amount of β-cells in islets, the concentration of immunoreactive material in them, specific indexes of allocation of islets, β-cells and insulin per unit area using immunohistochemical assay. Results were processed by statistical application package. We used Student's t-test and Wilcoxon's w-test when appropriate.

Results and discussion. In normoglycemic SHR about 80 % of pancreatic islets are small islets, whereas in normotensive Wistar rats the portion of small islets is less than 45 %. In SHR rats we found the 1/3 decrease of β-cells in islets with area less than 1500 μm^2, and 2-fold decrease in islets with area 3500–7500 μm^2; decrease of specific amount of β-cells (12,4 % compared with Wistar) and insulin contain (3-fold compared with Wistar).

Conclusions. Formation of hereditary hypertension in SHR is accompanied by remodeling of the insular apparatus of pancreas with prevailing of small and middle-sized islets, 2-fold decrease of amount of pancreatic islets and 8-fold decrease of β-cells cell amount. In normoglycemic SHR we found a middle β-cells hypertrophy and increased concentration of insulin. Herewith the specific insulin contain is 3-fold less in hypertensive compared with normotensive rats due to decrease of β-cells cell pool.

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Особливості організації популяції бета-клітин підшлункової залози щурів лінії SHR зі спонтанною гіпертензією

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Проблема контролю пулу β-клітин у пацієнтів із гіпертонічною хворобою є актуальною, оскільки не виключено, що генетичні дефекти формування спадкової артеріальної гіпертензії можуть мати вплив на механізми підтримання клітинної маси ендокриноцитів підшлункової залози та призводити до порушення метаболізму глюкози та діабету. Крім того, до порушення адекватної секреції інсуліну підшлунковою залозою може призводити порушення цитоархітектоніки панкреатичних острівців, що може бути спровоковано локальними порушеннями кровообігу в пацієнтів з артеріальною гіпертензією.

Мета роботи – вивчити параметри розподілу панкреатичних острівців у підшлунковій залозі та схарактеризувати морфофункціональний стан β-клітин у гіпертензивних щурів SHR.

Матеріали та методи. У гістологічних зрізах підшлункової залози за допомогою імунофлюоресцентного методу виявляли інсулін, аналізували площу панкреатичних острівців, кількість β-клітин у них, концентрацію у клітинах імунореактивного інсуліну, питомі показники розподілу острівців, β-клітин та інсуліну на одиницю площі залози. Результати опрацювали за допомогою пакета статистичних програм, для оцінювання вірогідності різниці у групах використовували t-критерій Стьюдента та W-критерій Вілкоксона.

Результати. У нормоглікемічних гіпертензивних щурів SHR майже 80 % панкреатичних острівців підшлункової залози є маленькими острівцями, водночас як у нормотензивних щурів Wistar їхня частка становить менше ніж 45 %. У щурів лінії SHR виявлене зменшення кількості β-клітин на 1/3 в острівцях площею менше ніж 1500 мкм^2 і вдвічі – в острівцях із площею 3500–7500 мкм^2, зменшення в залозі питомої ваги β-клітин (12,4 % порівняно з показниками Wistar) і вмісту інсуліну (втричі порівняно з Wistar).

Висновки. Формування спадкової артеріальної гіпертензії в щурів лінії SHR супроводжується ремоделюванням інсулярного апарату підшлункової залози з домінуванням маленьких і середніх острівців, зниженням кількості панкреатичних острівців удвічі та кількості β-клітин – у 8 разів. У нормоглікемічних гіпертензивних щурів лінії SHR визначається помірна гіпертрофія β-клітин і збільшення концентрації в них інсуліну. При цьому внаслідок зниження пулу ендокриноцитів питомий вміст інсуліну приблизно втричі менший, ніж у нормотензивних щурів лінії Wistar.

Ключові слова: *β-клітини, інсулін, артеріальна гіпертензія.*

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Особенности организации популяции бета-клеток поджелудочной железы крыс линии SHR со спонтанной гипертензией

Т. В. Абрамова, Ю. М. Колесник

Проблема контроля пула β-клеток у пациентов с гипертонической болезнью является актуальной, так как не исключено, что наследственные генетические дефекты формирования артериальной гипертензии могут затрагивать механизмы поддержания

клеточной массы эндокриноцитов в поджелудочной железе и приводить к нарушению метаболизма глюкозы и диабету. Кроме того, к нарушению адекватной секреции инсулина поджелудочной железой может приводить нарушение цитоархитектоники панкреатических островков, что может быть спровоцировано региональными нарушениями кровообращения у пациентов с артериальной гипертензией.

Цель работы – изучить параметры распределения панкреатических островков в поджелудочной железе и охарактеризовать морфофункциональное состояние β-клеток у гипертензивных крыс линии SHR.

Материалы и методы. В гистологических срезах поджелудочной железы иммунофлюоресцентным методом выявляли инсулин; анализировали площадь панкреатических островков, количество в них β-клеток, концентрацию в клетках иммунореактивного инсулина, удельные показатели распределения островков, β-клеток и инсулина на единицу площади железы. Результаты обрабатывали пакетом статистических программ, для оценки достоверности различий в группах применяли t-критерий Стьюдента и W-критерий Уилкоксона.

Результаты. У нормогликемических гипертензивных крыс линии SHR около 80 % панкреатических островков поджелудочной железы представлены маленькими островками, тогда как у нормотензивных крыс линии Wistar их доля составляет менее 45 %. У крыс линии SHR выявлено уменьшение численности β-клеток на 1/3 в островках площадью менее 1500 мкм^2 и в 2 раза в островках площадью 3500–7500 мкм^2, снижение в железе удельного количества β-клеток (12,4 % от показателя крыс линии Wistar) и содержания инсулина (в 3 раза по сравнению с крысами линии Wistar).

Выводы. Формирование наследственной артериальной гипертензии у крыс линии SHR сопровождается ремоделированием инсулярного аппарата поджелудочной железы с доминированием маленьких и средних островков, снижением количества панкреатических островков в 2 раза и уменьшением численности β-клеток в 8 раз. У нормогликемических гипертензивных крыс линии SHR отмечается умеренная гипретрофия β-клеток и повышение концентрации в них инсулина. При этом за счёт снижения пула эндокриноцитов удельное содержание инсулина примерно в 3 раза меньше, чем у нормотерзивных крыс линии Wistar.

Ключевые слова: *β-клетки, инсулин, артериальная гипертензия.*

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Essential hypertension and diabetes mellitus are widespread diseases with different etiology and pathogenesis. Nevertheless, comorbid pathogenicity is the characteristic for both nosologies [1], and is the most pronounced in patients with the metabolic syndrome. It is known, that long duration of essential hypertension leads to chronic disorders of the central and peripheral circulation, resulting in violation of microcirculation in different organs. It is not excluded that the pancreas, including its endocrine pancreatic islets, may be one more target organ damaged in hypertension. The pancreatic β-cells are responsible for the production of all insulin for maintaining the body glucose homeostasis. Circulatory disorders in the pancreas in arterial hypertension can negatively influence the mechanisms of β-cells regeneration [2], amount of secreted insulin and consequently promote glucose metabolism disturbance and diabetes development. Previously, we have shown [3] that the population of mature adult SHR rats with developed arterial hypertension can be divided into three approximately equal groups according to the basal level of glucose: 1) the animals with normal fasting plasma glucose level; 2) animals with fasting normoglycemia, but abnormal glucose tolerance; 3) animals with fasting hyperglycemia. Based on this, we assume that the formation of genetic hypertension may violate the physiological mechanisms of β-endocrinocytes mass control in the pancreas of SHR.

The **aim** of the study was to determine the parameters of pancreatic islets distribution in the pancreas and to characterize morphological and functional state of β-cells in SHR.

Materials and Methods

The research was carried out on 10 male normotensive Wistar rats (BP=105.0±1.1 mm Hg.; weight 232±7g; the level of fasting plasma glucose 3.94±0.09 mmol/l) and 15 SHR (BP=155.7±0.9 mm Hg.; weight 306±5g) with fasting normoglycemia (4.73±0.10 mmol/l). The animals were kept in standard conditions of the vivarium with natural lighting without water and food restrictions. Glucose concentration was determined by the glucose oxidase method and insulin by immunoassay (ELISA) in blood taken from the tail vein of the experimental rats.

The pancreas was removed after decapitation of experimental animals by thiopental anesthesia (50 mg/kg) and fixed in Bouin's solution (20 hours), and fixed in paraplast after standard histological processing (MkCormick, USA). The rehydrated serial histological sections of 5 μm thickness were detected by immunofluorescence method using insulin kit (Peninsula Lab. Inc., United Kingdom). Study of immunofluorescence reaction was carried out using a fluorescent microscope AxioImager-M2 (Carl Zeiss, Germany), equipped with a camera AxioCam-5HRm (Carl Zeiss, Germany), using emission light filter 38HE (λ_{ex}=470/40 nm, λ_{em}=525/50 nm) (Carl Zeiss, Germany). Quantitative analysis of immunofluorescence reaction was carried out using digital image analysis system AxioVision-4.8.2 (Carl Zeiss, Germany). Pancreatic islets are classified depending on their cross sectional area [4]. The concentration of immunoreactive insulin in the β-cells was calculated as the logarithm of the ratio of secretory granules fluorescence intensity to the non-specific fluorescence of acinar gland tissue and was presented in units of immunofluorescence (U_{if}). The insulin content in the pancreas was calculated as multiplication of insulin concentration, the area of the immunoreactive material in the cell, and relative number of β-cells (taking into account the different types of islets presence). This index was presented in U_{if}/cm^2 of gland slice area. The total examined area of pancreatic slices from each animal was at least 5 cm^2.

The experimental data were processed using software package for statistical analysis EXCEL 2003 (Microsoft

Corp.) with integrated software superstructure AtteStat. Data with the continuous distribution were presented as mean and SEM (M±m), and discretely distributed data (the number of cells, islets) – as median (Me) and interquartile range (Q1÷Q3). The significance of differences between experimental groups was evaluated using Student's t-test (for continuous data and distributed with a normal distribution), and Wilcoxon-W-test (for discretely distributed data), assuming reliable differences if the P-value is less than 0.05.

Results and Discussion

Comparative analysis of the pancreatic islets distribution in the pancreas of experimental animals evidenced that the number of islets in SHR is 2 times less than in normotensive Wistar rats (*Table 1*).

The significant differences in the various types of islets distribution were noted: normotensive rats represented 75 % of small (area less than 1500 μm^2) and middle (area 1500–3500 μm^2) islets in approximately equal proportions, but in hypertensive rats about 80 % of all islets were small. The pancreas of the SHR revealed a large number of single β-cells (9.7±1.1 % against 0.04±0.006 % in Wistar rats) and absence of islets with area more than 7500 μm^2. This pattern of pancreatic islets distribution in adult SHR is largely consistent with the picture, which was observed in 1-month Wistar rats with a physiological gestation [4] (in this period the embryonic type of animals β-cells changes to adult type [5]), as well as it was observed in adult experienced chronic prenatal stress Wistar rats [4].

The distinctive feature of SHR was the number of β-cells decreasing in cross section of pancreatic islets. This feature was the most notable in small islets, where the number of β-cells was 1/3 less than in Wistar rats, and in large islets, where the number of endocrine cells was 2-fold reduced (*Table 2*).

Features of the different types of pancreatic islets frequency distribution with reduced β-cells quantity resulted in fact that the number β-endocrinocytes in the pancreas of SHR was 12.4±0.1 % of the number of β-cells in Wistar rats. Moreover β-cells of large islets in SHR were larger ($p<0.001$), and immunoreactive insulin concentrations in them was 2.2 times higher than in Wistar rats. However, reduction of the β-cells number in SHR's pancreas resulted in 3 times decrease in the relative content of immunoreactive insulin in the pancreas compared with Wistar rats (*Table 1*).

A number of studies have noted that hypertension formation in SHR was accompanied by reduction of β-cells pool which was progressed with aging [6]. It differs significantly from insular apparatus changes in normotensive Wistar rats, as these animals showed significant reduction

Table 1

Parameters of pancreatic islets with β-cells distribution in pancreas (per 1 cm^2 of slice area) in Wistar (numerator) and SHR (denominator)

Islets Type	Number of Islets, Me (Q1÷Q3)	Number of Cells, Me (Q1÷Q3)	Insulin Content, U_{if}, M±m
Single β-cells	3 (3÷5) 14 (7÷15)$^{\#}$	5 (4÷7) 15 (10÷17)$^{\#}$	18.9±0.2 52.0±0.6*
Small (area<1500 μm^2)	98 (75÷131) 88 (66÷102)	556 (502÷837) 380 (296÷445)$^{\#}$	534.3±2.7 459.5±2.2*
Medium (area 1500–3500 μm^2)	67 (61÷76) 11 (7÷15) $^{\#}$	563 (450÷664) 196 (155÷216)$^{\#}$	3211.7±7.0 1032.1±0.7*
Large (area 3500–7500 μm^2)	31 (21÷38) 3 (1÷6)$^{\#}$	2281 (1382÷2919) 238 (199÷282)$^{\#}$	194.3±5.2 25.7±5.1*
Giant (area >7500 μm^2)	22 (14÷32) 0	2790 (1144÷5224) 0	283.3±21.5 0
Totally (M±m)	231±3 112±1$^{\#}$	6738±174 833±8$^{\#}$	4242.5±4.1 1537.9±1.2*

Notes: – $p<0.05$ for Student's t-test (*) and for Wilcoxon-W-test ($^{\#}$).

Table 2

Pancreatic β-cells characteristic in Wistar (numerator) and SHR (denominator)

Islets Type	β-cells Number in Cross Sectional Islet's Area, Me (Q1÷Q3)	β-cell area, μm^2, M±m	Insulin Concentration in β-cell, U_{if}, mM±m
Single β-cells	1 (1÷1) 1 (1÷1)	84.4±2.9 84.6±3.6	59.62±1.72 66.39±4.12
Small (area <1500 μm^2)	6 (3÷9) 4 (2÷5) $^{\#}$	91.6±0.9 106.8±7.8 *	18.62±0.78 23.85±1.04 *
Medium (area 1500–3500 μm^2)	22 (19÷24) 18 (14÷23) $^{\#}$	105.6±1.8 112.4±8.8	4.68±0.13 5.67±0.67
Large (area 3500–7500 μm^2)	81 (53÷83) 42 (34÷48) $^{\#}$	88.2±1.9 123.4±6.8 *	1.77±0.12 4.08±0.16 *
Giant (area >7500 μm^2)	136 (89÷183) 0	92.2±1.6 –	1.40±0.01 –

Notes: – $p<0.05$ for Student's t-test (*) and for Wilcoxon-W-test ($^{\#}$).

of β-endocrinocytes pool from the age of 18 months (aging animals) [7,8]. It is believed that reduction of β-cells mass in normotensive Wistar rats pancreas may be caused by regulatory β-cells mass controlling factors activity weakening due the animal's aging. Important regulators of β-cells proliferation are connective tissue growth factor (CTGF), parathyroid hormone-related protein (PTHrP) and glucagon-like peptide-1 (GLP-1) [9,10]. Reducing of their formation in the adult or in pathological conditions can decrease the degree of β-cells pool self-renewal. As pathogenic factors that inhibit β-cells proliferation can be a violation of blood flow and microcirculation in pancreatic islets in SHR [11], and islets sympathetic innervation changes [12,13], which can be formed as a total nervous dysregulation in hypertension [14]. The basis of β-cells mass reduction in the pancreas may be epigenetic mechanisms: it has been shown the decreasing in Bmi1 protein synthesis with aging, which inhibits the activity of *Ink/Arf* gene locus, and it leads to increased formation of β-endocrinocytes division inhibitor – protein $p16^{Ink4a}$ [15].

Nevertheless, as it has been shown in our studies, a decrease in the number of β-cells in SHR pancreas was largely compensated by higher concentrations of immunoreactive insulin in endocrine cells, which probably ensured the maintaining of immunoreactive insulin sufficient level in plasma (10.99±0.37 μIU/ml against 8.61±0.41 μIU/ml in Wistar rats) and fasting euglycemic indices. Of course, it is necessary to pay attention to the fact that adequate plasma insulin concentration maintaining in SHR was on the background of 3 times decreased indicator of relative immunoreactive insulin content in pancreas (*Table 1*). These results in our opinion are not contradictory, but rather show a high intensity of insulin processing and their secretion by β-cells into peripheral blood. On the one hand, it characterizes the high level of β-endocrinocytes compensatory capacity in their pool reducing in the SHR pancreas. On the other hand it could cause β-cells exhaustion due to long period of their functional overload. Taking into account that plasma lipids, triglyceride and cholesterol concentration are higher in SHR, compared with Wistar rats [3,16], it should be assumed that normoglycemic status of hypertensive rats should not be regarded as an indicator of carbohydrate homeostasis physiological state in these animals, because presence of lipid metabolism disorders may further contribute to glucose tolerance impairment and metabolic syndrome development.

Conclusions

1. Hereditary hypertension development in SHR is accompanied by the remodeling of pancreas insular apparatus with the dominance of small and medium-sized islets, reduction in the number of pancreatic islets in a 2-fold and an 8-fold decreasing in the number of β-cells.

2. In normoglycemic hypertensive SHR-line rats moderate hypertrophy of β-cells and increase of insulin concentrations in them are indicated. At the same time the relative insulin content is about 3-fold less than in normotensive Wistar rats due to endocrynocytes pool reduction.

Conflicts of Interest: authors have no conflict of interest to declare.

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