Age dynamics of strain differences in the morphofunctional state of pancreatic beta- and amylin-producing cells in SHR and Wistar rats

T. A. Hrekova*[A,B,C,D,F], O. V. Melnikova[B,C,E], Ye. V. Kadzharian[B,E]

Zaporizhzhia State Medical and Pharmaceutical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Large-scale epidemiological studies have shown that cardiac pathology and progressive atherosclerosis in patients with diabetes mellitus occurred already at the stage of prediabetes. Obesity and insulin resistance affect cardiometabolic health due to pleiotropic effects of insulin. Despite the vast range of research, some aspects remain hidden links in the overall pathogenesis of metabolic and hemodynamic disorders.

The aim of the work was to study the morphofunctional state of pancreatic islets (PIs), beta- and amylin-producing cells in male rats of Wistar strain (normotensive) and SHR (with spontaneous development of hypertension) in age dynamics.

Materials and methods. The study was carried out using 38 male Wistar rats and SHRs aged 7 and 24 months. Non-invasive blood pressure (BP) detection procedures were done using the BP-2000 Blood Pressure Analysis System. The morphofunctional state of PIs was examined in serial 5-μm thick pancreatic tissue sections. Beta- and amylin-producing cells were detected after histological preprocessing and the use of monoclonal FITC-conjugated antibodies. Image file processing was done via ImageJ software (National Institutes of Health, USA). Levels of glycemia were monitored with a SUPER GLUCOCARD-II glucometer.

Results. SHRs were hyperglycemic both at 7 and at 24 months, 8.41 ± 0.15 mmol/l and 8.90 ± 0.14 mmol/l, respectively, with elevated BP, 155 ± 5 / 80 ± 5 mm Hg and 165 ± 5 / 90 ± 5 mm, respectively. Old SHRs developed PI hypertrophy mainly associated with the increased number and percentage of beta-cells, apparently in response to hyperglycemia. Both in the PIs of adult and old SHRs, the number of amylin-producing cells was lower while the content of amylin was higher than those in the age-matched Wistar rats.

Conclusions. Male SHRs are characterized by a persistent increase in blood pressure and abnormalities of carbohydrate metabolism already at adult age, one of the manifestations of which is hyperglycemia worsening with age. Chronic hyperglycemia in SHRs due to the higher insulin requirement finds its expression in low content of this hormone in the islets at adult age and decreased its content in beta-cells in old animals.

Key words: pancreatic islets, hypertension, insulin, amylin, Wistar rats, SHR.

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Вікова динаміка штамових відмінностей морфофункціонального стану бета- та амілін-продукувальних клітин підшлункової залози у щурів SHR і Wistar

Т. А. Грекова, О. В. Мельнікова, Є. В. Каджарян

Масштабні епідеміологічні дослідження довели, що серцева патологія та атеросклероз, який прогресує, у хворих на цукровий діабет наявні вже на стадії переддіабету. Ожиріння й інсулінорезистентність впливають на кардіометаболічний стан через плейотропну дію інсуліну. Незважаючи на широкий спектр досліджень, деякі аспекти залишаються прихованими ланками поєднаного патогенезу метаболічних і гемодинамічних порушень.

Мета роботи – вивчити морфофункціональний стан панкреатичних острівців (ПО), бета- та амілін-продукувальних клітин у щурів-самців лінії Wistar (нормотензивних) та SHR (зі спонтанним розвитком гіпертензії) у віковій динаміці.

Матеріали та методи. Дослідження здійснили на 38 самцях щурів Wistar і SHR віком 7 і 24 місяці. Неінвазивне вимірювання артеріального тиску (АТ) виконали за допомогою системи аналізу АТ BP-2000. Морфофункціональний стан ПО дослідили на серійних зрізах тканини підшлункової залози завтовшки 5 мкм. Бета- та амілін-продукувальні клітини ідентифікували після попередньої гістологічної обробки та використання моноклональних FITC-кон'югованих антитіл. Файли зображень обробили засобами програмного забезпечення ImageJ. Рівні глікемії контролювали за допомогою глюкометра SUPER GLUCOCARD-II.

Результати. SHR були гіперглікемічними і через 7 (8,41 ± 0,15 ммоль/л), і через 24 місяці (8,90 ± 0,14 ммоль/л), мали підвищений АТ – 155 ± 5 / 80 ± 5 мм рт. ст. і 165 ± 5 / 90 ± 5 мм відповідно. У старих SHR розвинулася гіпертрофія ПО, насамперед пов'язана зі

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***E-mail:** grekovata@gmail.com

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збільшенням кількості та відсотка бета-клітин, імовірно, у відповідь на гіперглікемію. У ПО дорослих і старих SHR кількість клітин, що продукують амілін, менша, а вміст аміліну вищий, ніж у щурів лінії Wistar відповідного віку.

Висновки. Самці SHR характеризуються стійким підвищенням артеріального тиску та порушеннями вуглеводного обміну вже в дорослому віці, один із проявів цього – прогресування гіперглікемії з віком. Хронічну гіперглікемію у SHR внаслідок підвищеної потреби в інсуліні визначили за низьким вмістом цього гормона в острівцях у дорослому віці та зниженням його вмісту в бета-клітинах у старих тварин.

Ключові слова: панкреатичні острівці, спонтанна гіпертензія, інсулін, амілін, щури Wistar, SHR.

Актуальні питання фармацевтичної і медичної науки та практики. 2023. Т. 16, № 3(43). С. 244-248

Lately, a considerable number of information has been accumulated about arterial hypertension (AH) not only as a hemodynamic phenomenon, but rather as a complex syndrome that includes various combinations of abnormal distribution of adipose tissue, hyperactivity of the sympathetic nervous system, imbalance of pressor-depressor mechanisms, metabolic perturbations, and other abnormalities [1].

Large-scale epidemiological studies have shown that cardiac pathology and progressive atherosclerosis in patients with diabetes mellitus (DM) occurred already at the stage of prediabetes [2,3].

It is noteworthy that hypertension and dyslipidemia as traditional risk factors for cardiovascular complications are often documented in persons at the prediabetic state [4]. Intensive study results on complex and even contradictory mechanisms, by which obesity and insulin resistance (IR) affect cardiometabolic health, have the potential to extend beyond the earlier concepts existed over the years about IR as being associated exclusively with inadequate insulin effects on carbohydrate metabolism. The actual experience suggests pleiotropic effects of insulin involved in protein and fat metabolism, ion and amino acid transport, cell cycle regulation, cell proliferation and differentiation, and, not least, nitric oxide synthesis [5].

Initial interest in the hormone amylin as a major component and cause of islet amyloidosis prompted a series of intensive studies, resulting in the presentation of a 37-amino acid polypeptide produced and co-secreted with insulin by pancreatic beta-cells. Subsequently, experimental evidence was obtained for the synergistic regulation of blood glucose levels by insulin and amylin, the genes of which contain similar promoter elements, and the transcription factor PDX1 regulates the effect of glucose on both genes [6]. However, the parallel pattern of glucose-stimulated insulin and amylin secretion by beta-cells was altered in experimental models of diabetes, indicating that strict co-expression of the genes for these hormones may be disrupted under certain conditions.

Team collaboration between clinicians and morphologists, having a shared interest to identify the mechanisms of the onset and progression of so prevalent comorbid pathology, has demonstrated the direct effect of hyperamylinemia with the impaired pancreatic beta-cell function on the development of diastolic dysfunction and myocardial hypertrophy after the onset of type 2 DM clinical course, as well as on aggravation of the latter [7,8].

Given the immediacy of the issue, the incidence and complications of this pathology, research has continued at all levels, including both experimental modeling and clinical studies. Modern approaches to simulation of AH and DM in animals are based on several methodologies. First, functional or morphological abnormalities in disease models induced by characteristic milieu conditioning or specific agent administration; second, the use of genetic animal strains, for example, spontaneously hypertensive rats (SHR) that are primarily normotensive and spontaneously develop chronic hypertension as they age [9].

Despite the vast range of the already carried out and continuing scientific research, some aspects remain hidden links in the overall pathogenesis of metabolic and hemodynamic disorders.

Aim

Therefore, the aim of the work was to study the morphofunctional state of pancreatic islets (PIs), beta- and amylin-producing cells in male rats of Wistar strain (normotensive) and SHR (with spontaneous development of hypertension) in age dynamics.

Materials and methods

The study was carried out using 38 male rats assigned to 4 groups: Groups 1 and 2 of 10 animals each – intact male Wistar rats at the age of 7 and 24 months; Groups 3 and 4 of 10 and 8 animals, respectively, – 7- and 24-month-old male SHR.

Experimental procedures of the study were carried out precisely following the national "General Ethical Principles of Animal Experiments" (Ukraine, 2001), aligned with the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" provisions (Strasbourg, 1986), "Statement on Animal use in Biomedical Research", adopted by the 41st World Medical Assembly (Hong Kong, September 1989) and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes.

Non-invasive blood pressure (BP) detection procedures were done twice using the BP-2000 Blood Pressure Analysis System (Visitech Systems, USA): prior to forming the groups and at the end of the experimental period, before the rats were anesthetized with thiopental (80 mg/kg body weight).

The morphofunctional state of PIs was examined in serial 5-μm thick sections from various parts of the pancreas. Beta- and amylin-producing cells were detected after histological

Table 1. The area of PIs, the number and area of beta- and amylin-producing cells, the content of insulin and amylin in the cells and PIs of SHR and Wistar rats in age dynamics (M ± m)

Study series	Wistar		SHR	
	7 months	24 months	7 months	24 months
Beta-cells	23 ± 2 / 58.83 ± 0.29	17 ± 2 / 69.99 ± 0.78(1)	7 ± 1 / 65.42 ± 0.32(1)	22 ± 3 / 66.97 ± 0.43(1*,2)
Amylin-producing cells	13 ± 1 / 58.67 ± 1.15	14 ± 1 / 48.15 ± 1.39(1)	3 ± 0 / 51.47 ± 0.49(1)	8 ± 1(2) / 57.18 ± 0.26(1*,2)
PI area, μm²	2137 ± 134	1610 ± 136(1)	845 ± 119(1)	3150 ± 410(1,1*)

In the numerator: the mean number of cells in the PI; **in the denominator:** the mean cell area; significance of differences $p < 0.05$ in comparison to 7-month-old(1) and 24-month-old(2) intact animals and 7-month-old SHRs(1*).

preprocessing and the use of monoclonal FITC-conjugated antibodies (kits for the immunofluorescence detection of insulin and amylin in tissues manufactured by Peninsula Laboratories Inc., USA). PIs were identified in the ultraviolet excitation spectrum at 390 nm with a high emission 38HE optical light filter (Carl Zeiss, Germany) under an AxioScope microscope (Carl Zeiss, Germany) using the AxioVision 40 V 4.8 software 2.0 (license No. 3005339). An interactive mode of image analysis included extracting an area with statistically significant fluorescence. At least 100 visual fields in each series were subjected to the study, followed by file processing via ImageJ software (National Institutes of Health, USA). For each PI found, parameters characterizing the concentration of hormones and their content in the islet were calculated, automatically detected morphometric parameters were studied: the PI area (μm²); the area of the immunoreactive material in the islet (μm²); the cross-sectional area and number of beta- and amylin-producing cells in the PI. Levels of glycemia were monitored with a SUPER GLUCOCARD-II glucometer (Arkray Factory, Japan).

The value of the arithmetic mean of the sample (M), its variance and the error of the mean (m) were calculated for all indicators. The statistical comparison of the study results was done by ANOVA. A value of $p < 0.05$ was considered to be statistically significant.

Results

The study revealed that SHRs were hyperglycemic both at adult age (7 months) and at old age (24 months), 8.41 ± 0.15 mmol/l and 8.90 ± 0.14 mmol/l, respectively, with elevated BP, 155 ± 5 / 80 ± 5 mm Hg and 165 ± 5 / 90 ± 5 mm Hg at the age of 7 and 24 months, respectively. In Wistar rats with stable BP levels (135 ± 5 / 75 ± 5 mm Hg at 7 and 24 months of age), hyperglycemia was observed only at the age of 24 months (6.07 ± 0.16 mmol/l), while it remained in the euglycemic range (4.71 ± 0.11 mmol/l) in adults.

An analysis of the immunohistochemical findings of PI morphofunctional state showed a predominance of small-sized PI in their structure classified by area either in male SHR or in Wistar rats, and their number was significantly higher in 7-month-old SHR than that in the age-matched Wistar rats, 80 % versus 51 %, respectively. In old Wistar males, only small- and medium-sized islets were identified, while large- and giant-sized ones were seen in the age-matched SHR males, that accounted for 12 % and 13 % of the PIs total number identified, respectively (*Table 1*).

A meaningful finding was that the mean area of PIs in old SHRs was 4 times ($p < 0.05$) higher compared to that in adult ones (*Table 1*), but this index was, on the contrary, 25 % ($p < 0.05$) lessened in Wistar rats by the age of 24 months. To specify parameters, due to which such a notable change in the mean area of PIs occurred in the animals, the mean number and area of beta- and amylin-producing endocrinocytes were analyzed in their composition. It turned out that the number of beta-cells in the PIs of old SHR was 3 times greater ($p < 0.05$) in comparison to 7-month-old rats, probably causing an increase in their area (*Table 1*).

Similarly, a 26 % decrease ($p < 0.05$) in this parameter in old Wistar rats compared to adult ones was the most probable reason for the PI area reduction. A comparison across rat lines showed that the mean number of beta-cells in the SHR PIs at 7 months was 70 % ($p < 0.05$) lesser, and at 24 months – 30 % ($p < 0.05$) higher as compared to the age-matched Wistar ones. Changes in the mean area of beta-cells in the experimental and control animals were not as significant, since this mean value was only 2 % more ($p < 0.05$) in old SHR and 19 % ($p < 0.05$) – in Wistar rats when compared with adult ones. Therewith, the mean area of beta-endocrinocytes in 7-month-old SHR was 11 % more ($p < 0.05$), and at 24 months, it was 4 % less ($p < 0.05$) than that in the age-matched Wistar rats (*Table 1*).

The mean content of insulin in beta-cells did not differ significantly when comparing male SHRs at the age of 7 and 24 months. Contrariwise, this value was significantly increased in old Wistar rats, almost 3 times ($p < 0.05$), compared to that in adults. Subsequent interstrain comparison of the mean insulin content in the beta-cells of animals showed that it was 17 % ($p < 0.05$) greater in SHRs at 7 months, but 60 % ($p < 0.05$) less at 24 months than that in the cells of the age-matched Wistar rats. With regard to the mean insulin content in the PIs, it exceeded the mean value of adult rats by 2.7 times ($p < 0.05$) in 24-month-old SHRs. The same occurred in old Wistar rats, as evidenced by an increase in this parameter by 1.6 times ($p < 0.05$) in comparison to 7-month-old animals. It is worth noting that the PIs of both adult and old SHRs contained less insulin than those of the

age-matched Wistar rats, by 65 % and 42 % ($p < 0.05$) at 7 and 24 months, respectively.

The PIs of 24-month-old SHRs were characterized by a higher mean number of amylin-producing cells (2.6 times, $p < 0.05$) as compared to 7-month-old ones. When quantitatively comparing this value with that of Wistar rats, the numerical data were clearly defined by 78 % and 43 % ($p < 0.05$) less at 7 and 24 months, respectively. Interestingly, the mean area of amylin-producing cells in SHRs was 11 % ($p < 0.05$) increased by the age of 24 months, being 12 % ($p < 0.05$) less at the age of 7 months, however, by 19 % ($p < 0.05$) more at 24 months, which was demonstrated by the results of comparison with the age-matched Wistar rats.

The mean content of amylin in the producing it cells of SHRs at 24 months became statistically significantly lower by 4 % ($p < 0.05$) when compared to that of adult animals. This parameter was changed in Wistar rats in an entirely different way, namely, it was 15 % ($p < 0.05$) increased by the age of 24 months. But the most noteworthy were the results of interstrain comparison, which revealed a significantly higher content of amylin in the cells producing it in SHRs both at 7 and 24 months, which in numbers were 35 % and 13 % ($p < 0.05$), respectively, from the indicators of the age-matched Wistar rats.

A comparative single-strain analysis of the mean amylin content in the PIs of adult and old animals showed a two-fold increase in this indicator ($p < 0.05$) in SHRs with no statistically significant changes in Wistar rats. However, particular emphasis was placed on the results of interstrain comparisons, strongly indicating the specific pattern of changes in the amylin content in the SHR PIs, since it was 40 % less ($p < 0.05$) at 7 months of age and became 50 % more ($p < 0.05$) at 24 months than that in the age-matched Wistar rats.

Age-specific differences in the percentages of the studied endocrinocytes in the PIs of Wistar and SHRs were determined. Thus, the percentage of beta-cells in adult and old SHRs was 70 % and 73 %, respectively, that was higher than that in the age-matched Wistar rats, representing 61 % and 50 %, respectively. So, the maximum percentage of amylin-producing cells (47 %) in 24-month-old SHRs made total sense within the context of the already obtained morphofunctional parameters.

Discussion

The pathogenetic links between type 2 DM, essential hypertension with quite a predictable pattern of hyperinsulinemia/IR and obesity development has got robust experimental evidence and been clinically confirmed, as well as the absence of any association between IR and secondary hypertension [10,11]. The competence of the widely used model for the study of essential hypertension on the strain SHR with genetically modified hypertension, manifesting left ventricular hypertrophy along with hyperinsulinemia/IR is worth mentioning [12,13].

The thrust of the study on multifactorial alterations of metabolic phenotypes is to reveal multiple neuronal, interhormonal associations and interreceptor coupling, as well as many other interactions, followed by the determination of intracellular activity. Even though the morphofunctional analysis to some extent represents a mechanistic approach, this study has revealed a number of structural and functional patterns of the conceptual relationship between impaired glucose metabolism and hypertension. Namely, in 7-month-old SHRs, the mean PI area was more than half less, and at 24 months, it was nearly double that of the age-matched Wistar rats. A four-fold increase in the mean PI area in old male SHRs appeared to be mostly driven by an increase in the beta-cell number. By contrast, in old Wistar rats, the mean PI area was reduced due to a decrease in the mean beta-cell number, despite their compensatory hypertrophy. Such alteration has been confirmed by the capacity of beta-cells to maintain metabolic homeostasis and normoglycemia through an increase in insulin secretion and/or a rise in the beta-cell number within the PIs [14,15].

In 24-month-old SHRs, instead, the number and area of amylin-producing cells was increased, thereby increasing the PIs area in these animals. Such a detection is in line with a broadly accepted viewpoint on the toxic effect of hyperamylinemia and the subsequent deposition of amyloid, causing beta-cell dysfunction and contributing to the development of type 2 DM as well as pathogenetically associated cardiovascular disorders [7,16].

However, both at 7 and at 24 months, SHR PIs were characterized by low number of amylin-producing cells, albeit increasing between the two ages. In this regard, it is quite appropriate to suggest a state of impaired carbohydrate metabolism even before the onset of fasting hyperglycemia in hypertensive animals, given the hypoglycemic effect of amylin as a central anorexigen and satiety signal associated with delayed gastric emptying, suppressed secretion of gastric juice and the contra-insular hormone glucagon. And most importantly, amylin is involved in the metabolism of adipose tissue, likewise leptin, regulating body weight in adults [17,18].

Summarizing the data presented on the resulting functional insufficiency of beta-cells in the formation of genetically determined AH, it is reasonable to assume their hyperstimulation by persistent hyperglycemia, followed by depletion with progression of IR, which closes the vicious circle of the metabolic syndrome.

Conclusions

1. Male SHRs are characterized by a persistent increase in blood pressure and abnormalities of carbohydrate metabolism already at adult age, one of the manifestations of which is hyperglycemia worsening with age.
2. The revealed hypertrophy of pancreatic islets in old SHRs is mainly associated with the increased number and percentage of beta-cells, that is an important compensatory mechanism in response to hyperglycemia.
3. Chronic hyperglycemia in SHRs due to the higher insulin requirement finds its expression in low content of this hormone in the islets at adult age and decreased its content in beta-cells in old animals.

4. Both in the pancreatic islets of adult and old SHRs, the number of amylin-producing cells is lower while the content of amylin is higher than those in the age-matched Wistar rats.

Conflicts of interest: authors have no conflict of interest to declare.
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Information about the authors:
Hrekova T. A., MD, PhD, Associate Professor of the Department of Pathological Physiology with the Course of Normal Physiology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0001-9946-3336
Melnikova O. V., MD, PhD, Associate Professor of the Department of Pathological Physiology with Course of Normal Physiology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-6807-8440
Kadzharian Ye. V., MD, PhD, Associate Professor of the Department of Pathological Physiology with Course of Normal Physiology, Zaporizhzhia State Medical and Pharmaceutical University, Ukraine.
ORCID ID: 0000-0002-3640-0749

Відомості про авторів:
Грекова Т. А., канд. мед. наук, доцент каф. патологічної фізіології з курсом нормальної фізіології, Запорізький державний медико-фармацевтичний університет, Україна.
Мельнікова О. В., канд. мед. наук, доцент каф. патологічної фізіології з курсом нормальної фізіології, Запорізький державний медико-фармацевтичний університет, Україна.
Каджарян Є. В., канд. мед. наук, доцент каф. патологічної фізіології з курсом нормальної фізіології, Запорізький державний медико-фармацевтичний університет, Україна.

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