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Reduction of the β-Cell Component of Pancreatic Islets in Spontaneously Hypertensive Rats

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Summary. A morphometric study of the pancreatic islets in young spontaneously hypertensive rats (SHR, Okamoto and Aoki Wistar strain) and in normotensive Wistar rats (NWR) of the same age revealed that the SHR (in prehypertensive and in early hypertensive stages) had a significantly smaller mass of islet tissue and that the number of islets in the SHR was reduced by half. The ratio between the total masses of the pancreatic islets for the NWR and the SHR at the prehypertensive and early hypertensive stages was found to be: 1:0.53:0.61, respectively. The mass of the islet tissue in the SHR was reduced at the expense of the β-cell component of the islets. No morphologic differences were found in the acinous tissue, and the pancreas and body weights were the same in both experimental groups.

The glucose tolerance test revealed reduced glucose utilization in the SHR, which may be due to a relative insufficiency of the insulin secretion by the islets upon rapid (i.v.) glucose lodaing. Reduced plasma insulin response to i.v. glucose loading and a reduced rate of insulin utilization were found in the SHR as compared with the NWR.

The poor development of the β-cell tissue may be attributed either to the specific effect of the enhanced catecholamine excretion or to a low insulin requirement of the cell tissue membranes (insulin targets) as a consequence of the membrane ion transport alteration in spontaneous hypertension.

Key words: Spontaneous hypertension — Pancreatic islets — Glucose tolerance test — Plasma insulin.

Most patients with essential hypertension have been found to exhibit some changes in the function of the pancreatic islets and in hydrocarbon metabolism (Welborn et al., 1966; Baumann et al., 1968, 1971), which may be assumed to indicate insufficiency of insulin production. Though it seems that this phenomenon does not play a significant role in the development of hypertension itself, it is, nevertheless, of certain interest, since the comprehension of its causes can throw more light on the nature of essential hypertension. The use of the strain of spontaneously hypertensive rats (Okamoto and Aoki), which presents the most adequate model of essential hypertension presently available makes accessible the study of the pancreatic islets at those early stages of hypertension when the chronic ischemia due to arteriolar and arterial sclerosis has not yet produced a secondary effect on the pancreatic tissues.

This paper reports the results of morphometric study of the pancreatic islets in young spontaneously hypertensive rats (SHR) at the prehypertensive and early

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hypertensive stages and normal Wistar rats of the same age. Furthermore, we have studied, in both experimental groups, glucose tolerance test results and the plasma insulin response following intravenous glucose injection.

Material and Methods

The animals used in our studies were the spontaneously hypertensive rats (SHR, Okamoto and Aoki Wistar strain) obtained from an inbred colony which has been maintained in our laboratory[1] since 1973. The control animals were inbred normotensive Wistar rats (NWR) obtained from the Stolbovaya breeding station of the USSR Academy of Sciences. Only male rats were used. All the animals were kept under identical conditions from the moment of weaning. Males aged 55–60 days were taken for morphometric studies of the pancreatic islets. The blood pressure at the moment of study ranged from 135 to 180 mmHg for some of the SHR (the early hypertensive stage) while other rats were still at the prehypertensive stage (70–105 mmHg). The blood pressure of the control NWR ranged from 70 to 100 mmHg.

A weekly measurement of the systolic blood pressure was made for all the animals starting from the age of 5 weeks and measurement was made a day before sacrifice. The blood pressure was determined under light ether anesthesia by tail plethysmography using a tail cuff and a photoelectric MPP-3 plethysmograph with MPP-3C transducer (Nihon Kohden). The plethysmogram and the tailcuff pressure were recorded with a polygraph. The pressure for each animal was determined as the mean of three consecutive measurements.

The rats were sacrificed by decapitation after a 10-h period of starvation, the pancreas was defatted by careful preparation, weighed, fixed in Bouin's fluid, and embedded in paraffin. Serial sections 5 μ thick were stained for β cells with aldehyde fuchsin according to Gömori and for α cells according to Paccini, as well as with phosphotungstic acid hematoxylin.

Following general histologic and histochemical study of the pancreas, the mean diameter of the pancreatic islets was determined using a screw ocular micrometer. For this, the greatest and least diameters of 100 islets were measured (in 5–6 sections for SHR and 3–4 sections for NWR, the distance between consecutive sections was 115 μ). The mean diameters of the α and β cells were determined (from measurements of the greatest and least diameters of 100 cells of each type), the numbers of α and β cells per 100 μ^2 of the islet's area were determined using a quadratic lattice incorporated into the eyepiece, and the ratio of the number of β/α cells was calculated (this was determined by counting the number of β and α cells in 10 islets, whose areas were measured).

The *number of islets* per 10 mm^2 of pancreatic tissue and the percentage of islet tissue area in terms of the total pancreatic area were determined from sections using a planimetric technique on drawing projections of tissue sections stained with phosphotungstic acid hematoxylin. We felt that it was preferable to count separately the relatively small β-cell islets, which some authors classify as less differentiated structures than α- and β-cell islets (Baranov et al., 1973).

The *histochemical method of Voigt* was used to demonstrate Zn content in the pancreatic islets in SHR (3 rats aged 12 weeks, blood pressure 140–160 mmHg) and in 3 NWR; these animals were also starved for 10 h prior to sacrifice.—The *numbre of animals* used in the morphometric studies is given in Table 1.

The *glucose tolerance test* and determination of plasma immunoreactive insulin (IRI) after intravenous injection of glucose were carried out separately for the male SHR and the NWR aged 12 weeks. All the SHR used in this experiment had an arterial pressure of 140–160 mmHg. Before testing the rats were starved for 10 h, with free access to tap water. The tests were carried out under deep nembutal anesthesia. Intravenous injection of 40% glucose was carried out for 5 s (150 mg of glucose per 100 g body weight). Blood samples (0.2 ml for the glucose and 0.5 for the IRI) were taken from the jugular vein before and at 10, 25, 45, and 60 min after glucose injection.—The *hematocrit level* at the begining and the end of the experiment did not change, indicating good compensation for the blood loss.—The *blood glucose*

1 Dr. Jacques Genest, Clinical Research Institute of Montreal, kindly supplied the spontaneously hypertensive rats

Table 1. Autopsy data and morphometric characteristics of the pancreatic islets in spontaneously hypertensive rats and in normotensive Wistar rats

Group	No. of rats	Systolic blood pressure (mmHg)	Weight		Mean diameter of islets (μ)	Islet tissue (%)	Quantity of islets for 10 mm² of pancreatic section		Quantity of cells for 100 μ² of islet area		β/α	Mean diameter of nucleus (μ)	
			Body weight (g)	Pancreas (mg)			α- + β-cell islets	β-cell islets	α cells	β cells		α cell	β cell
SHR (hypertensive stage)	10	141 ± 5.0	97.0 ± 1.3	196 ± 12.2	102.8 ± 3.5	0.67 ± 0.06	6.8 ± 0.8	0.6 ± 0.06	0.18 ± 0.03	0.21 ± 0.02	1.2	5.1 ± 0.08	5.1 ± 0.04
SHR (prehypertensive stage)	10	77 ± 6.0	91.0 ± 4.2	181 ± 9.3	89.5 ± 2.4	0.54 ± 0.04	6.5 ± 0.4	1.0 ± 0.2	0.22 ± 0.02	0.23 ± 0.01	1.2	4.9 ± 0.06	5.0 ± 0.07
Normotensive Wistar rats	10	77 ± 4.0	94.0 ± 3.5	196 ± 12.4	83.7 ± 2.6	1.1 ± 0.1	12.5 ± 0.9	5.6 ± 0.6	0.09 ± 0.008	0.27 ± 0.01	3.2	4.8 ± 0.06	4.9 ± 0.07
P_{1-2}		< 0.001	*	*	< 0.01	<*	*	<*	*	*		*	*
P_{1-3}		< 0.001	*	*	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001	< 0.01		*	*
P_{2-3}		*	*	*	*	< 0.001	< 0.001	< 0.001	< 0.001			*	*

* Not significant

levels were measured with an Autoanalyzer (Technicon, USA) by the cupric-neocuprioine chelate method. The plasma IRI levels were determined using a standard insulin radioimmunoassay kit (CEA-IRE-SORIN Association). Prior to the experiment we had found that the analytical system used in the kit had almost the same binding ability for the rat's plasma insulin as for the human standard insulin: in our experiments the binding ability of the standard human insulin varied from 59 to 23% according to the calibration curve, while the binding ability of the rat's plasma insulin lay within the range 58–32%. Results were presented as mean $\pm$ S.E. The statistical significance of the differences between group means was assessed by Student's "t" test. P values less than 0.05 were considered significant.

Results

There were practically no differences between the two groups of rats in body weight and absolute weight of the pancreas. However, the SHR had somewhat heavier adrenal glands, hearts, and hypophyses, as was also observed after comparing them with normotensive Wistar Kyoto rats (Okamoto, 1969).

a) Histologic and Morphometric Study

The general histologic examination of the pancreatic acinous tissue revealed no significant differences between the animal groups studied. However, the spontaneously hypertensive rats (both at the prehypertensive and the early hypertensive stages) showed a number of changes in the islets.

Firstly, the SHR were found to have a greatly reduced number of islets per unit area of the pancreatic section, as compared to normotensive Wistar rats (Table 1).

If the number of the β-cell islets is calculated separately, the difference becomes even more striking: such islets make up almost a half of all the islets observed on the histologic sections from the normotensive Wistar rats of the control group while for the SHR such islets constitute only 10% of the total number of islets. The SHR have twice as many α cells per 100 mm^2 of the islet's cross-section area in the islets which contain both α and β cells, while the number of the β cells is, on the contrary, reduced in comparison to the normotensive Wistar rats. Thus, the cell number ratio β/α in these islets for normotensive Wistar rats is three times that of the SHR. Even though the mean diameter of the islets in the SHR is somewhat larger than in the normotensive Wistar rats (this is clearly shown by comparing the early hypertensive SHR with the control animals), both SHR groups had a considerably lower percentage of total islet tissue area on the histologic sections than the Wistar rats (SHR: 0.67 and 0.54%; NWR: 1.1%). According to Delesse, the fraction of the inclusion area on a tissue section equals the fraction of this inclusion volume and hence we could determine the relative mass of islet tissue in the pancreas (proceeding from the weight of the pancreas and assuming that the specific weights of the acinous and islet tissue are the same in all the experimental groups). These calculations yielded the following ratio between the total masses of the pancreatic islets for the normotensive Wistar rats and for the SHR at the prehypertensive and early hypertensive stages: 1:0.53:0.61, respectively (Fig. 1).

No significant differences between the groups studied were found in measurements of diameters of the β-and α-cell nuclei. No dystrophic or atrophic changes were observed in the β cells, though the SHR exhibited a weaker zinc reaction and a considerably reduced quantity of Gömori-positive granulation in the β cells of

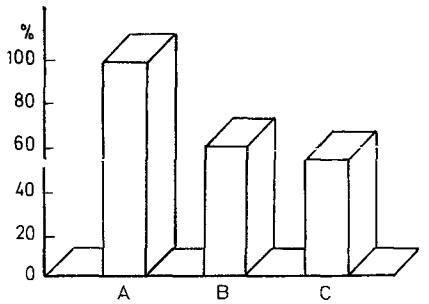
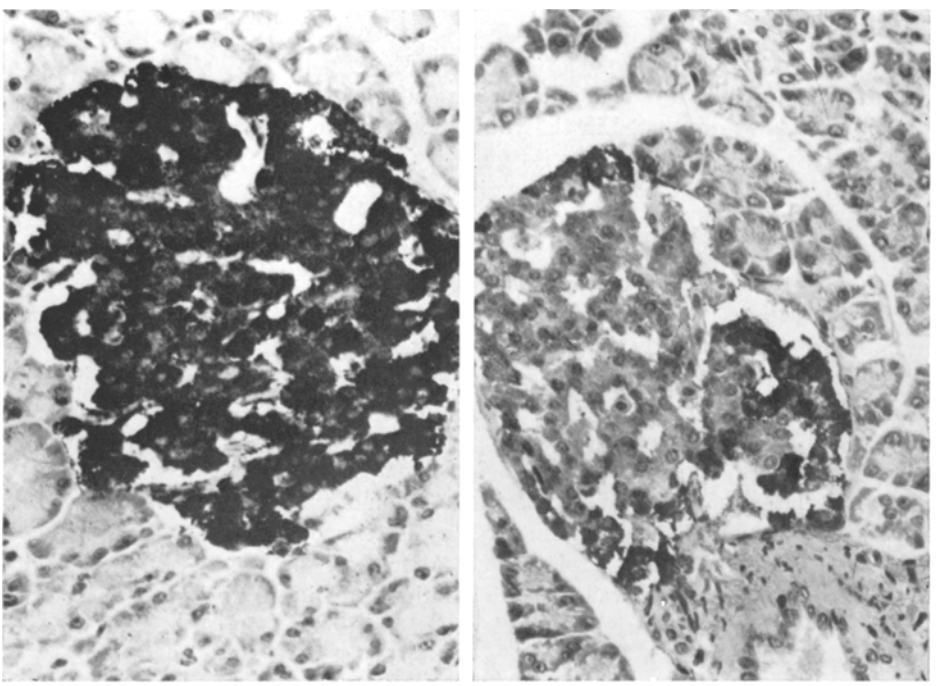


Fig. 1a—c. Relative mass of islet tissue in pancreas of spontaneously hypertensive rats and normotensive Wistar rats. (a) Normotensive Wistar rats. (b) SHR (early hypertensive stage). (c) SHR (prehypertensive stage)



a b

Fig. 2a and b. Zn content in Langerhans islets. (a) Normotensive Wistar rat. (b) spontaneously hypertensive rat. Voigt sulfide silver method. ×240

the islets, as compared to the normotensive Wistar rats. The zinc content in the α cells of the SHR, however, remained high (Fig. 2).

b) The Glucose Tolerance Test and the Plasma Immunoreactive Insulin Levels before and after Intravenous Glucose Injection

After a 10-h period of starvation the SHR and the normotensive Wistar rats had the same blood glucose levels (75.0 ± 2.4 mg% and 73.0 ± 2.2 mg%, respecti-

vely). The peak blood glucose level following injection was the same in both groups, but after 60 min the blood glucose level in normotensive Wistar rats returned close to the normal values, while the glucose level in SHR remained high (Fig. 3b).

Prior to glucose injection the plasma IRI level in SHR was lower than in the normotensive Wistar rats (14.0 ± 1.0 μU/ml vs. 33.0 ± 2.0 P/ml, $\mu U < 0.001$, Fig. 3a). The samples taken 10 min after glucose injection revealed higher insulin levels for both groups, but the peak for the SHR was not as high as for the normotensive Wistar rats. In the NWR the plasma IRI level, 25 min after injection, was below the initial one and dropped significantly at 60 min (from 33.0 ± 2.0 μU/ml before and 5.0 ± 1.0 μU/ml at 60 min after glucose injection). The plasma IRI level at 25 min after glucose injection was considerably higher in the SHR than the initial one and there was no drop of plasma insulin concentration at 60 min in the SHR such as was seen in the NWR.

Discussion

Our results show that the β-cell component of the pancreatic islet tissue is considerably less developed in the young SHR at the prehypertensive and early hypertensive stages, as regards its quantity, than in the normotensive Wistar rats. This is concluded from the experimental findings: an almost 50% decrease in the islets tissue mass and a 50% decrease in the number of islets in the SHR as compared to the control group of the normotensive Wistar rats, though no differences were found in the pancreas weight and the morphologic data for the acinous tissue between the rats of these groups. The observed decrease in the number of islets is chiefly due to the β-cell islets whose structural differentiation is not as marked as that of the islets containing both α and β cells. In the latter islets the ratio of the number of cells β/α was shifted to an increase of α cells. Hence, this change of the pancreatic islets observed in the SHR is, definitely, due to the morphologic adaptation of the β-cell component of the islets which seems to be caused by inhibition of the specific function of the β cells. There is no quantitative change of the α component of the islet tissue.

The results of the glucose tolerance test for the SHR are in good agreement with the data on the reduction of the insulin-producing component of the pancreatic islet tissue. A relative insufficiency of insulin secretion by the islets after rapid massive glucose loading may account for the slower glucose utilization strikingly exhibited by SHR as compared to normotensive Wistar rats. This conclusion also seems to be supported by the relatively small insulin response observed at the 10th min after glucose infusion, which can, to a certain extent, characterize the rate of release of insulin from the islets into the blood. The histochemical determination of zinc also indicated a somewhat smaller zinc content in the β cells of the islets (Fig. 2). This may be explained either by an impaired insulin production by the β cells or by a relatively enhanced insulin secretion by the islets, whose total mass is decreased in the SHR.

The observed differences in the islet tissue of the SHR and the normotensive Wistar rats of our control group should not be regarded as deviations of the pancreas structure in the different populations of the Wistar line, even though no absolute control group, that is the normotensive Wistar Kyoto rats, was available

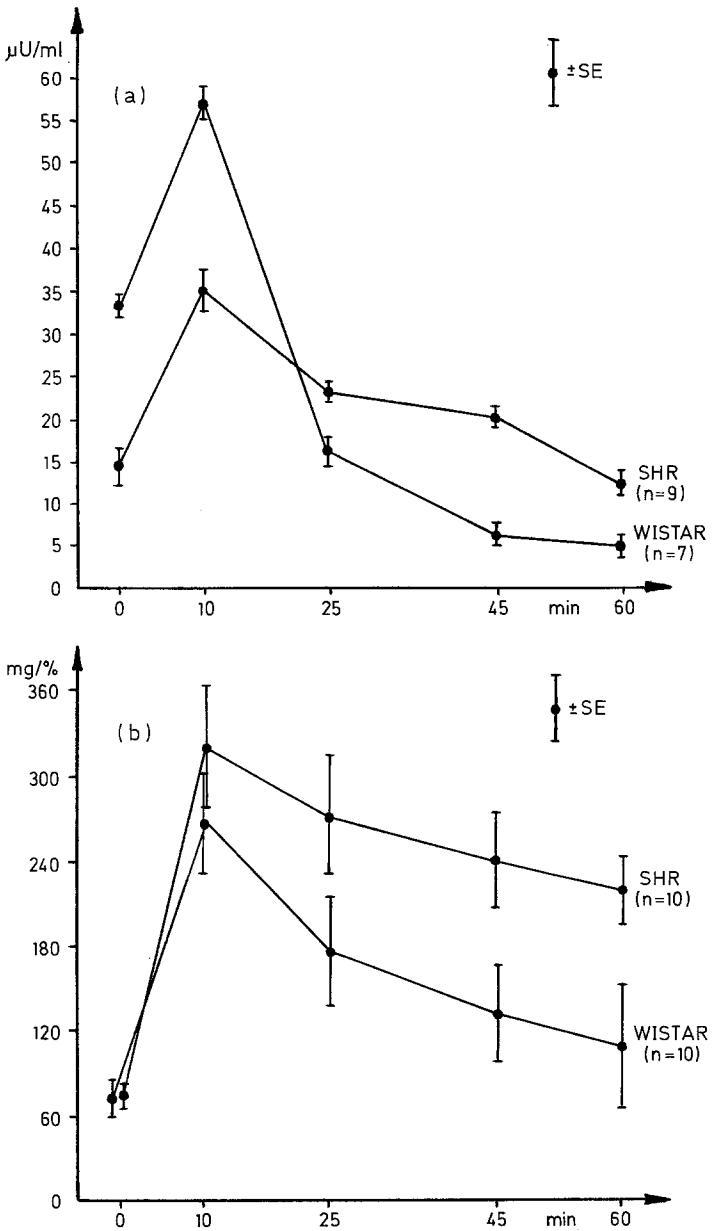


Fig. 3a and b. Plasma immunoreactive insulin (*a*) and glucose (*b*) content after intravenous glucose injection. *n* refers to number of animals. Mean values ± S. E. given

in our experiments. The quantitative reduction of the β-cell component of the islets in the SHR may be classified among other observed morphologic features which are caused, directly or indirectly, by the genetic pathology characteristic of these animals, such as hyperplasia and hypertrophy of the basophils of adeno-

hypophysis, hypertrophy of the adrenal cortex, or hypertrophy of the neurons of the macrocellular neurosecretory hypothalamic nuclei (Okamoto, 1969; Fukushima, 1968) .This statement does not mean that both characteristics studied (the quantitative reduction of the β-cell component of the islets and the arterial hypertension) are interrelated at the cellular genetic level. The decrease in mass of the insulin-producing tissue does not seem to belong to the primary manifestations of the genetic pathology but, rather, is secondary in character: it may be caused by lower insulin requirement of the tissue, primarily, the cell tissue membranes. The latter effect might be caused by a factor which alters the insulin tolerance of the cell membrane or simulates in some way the action it exerts on the transmembrane transport of substances. Such a factor may be an alteration of the ion membrane permeability, which is either genetically determined or has a secondary origin. This assumption is supported to a certain extent by the data reported by Jones (1973), who found a selective increase in the potassium permeability of the membrane of arterial smooth muscle in young SHR. Further support is given by the finding of considerably higher sodium and potassium passive permeability of the erythrocyte membrane in the SHR compared to the normotensive Wistar and Sprague-Dawley rats (Postnov et al., 1975a, b). These alterations of membrane ion permeability do not seem to be directly associated with the corticosteroid effect, since they are fully retained in animals who had been previously adrenalectomized. If these findings present a partial indication of a more widespread defect in the cell membrane permeability in the SHR, we have some grounds to assume that the decrease in the mass of the insulin-producing tissue found in these animals is a remote consequence of this defect. Another possible explanation for the inhibitation of the specific function of the insulin-producing tissue in the SHR may be related to the well-known competition between the effects of insulin and catecholamines, which seems to play a significant role in the pathogenesis of the spontaneous hypertension.

It should be noted, however, that the behavior of the plasma insulin concentration plot following glucose injection indicates its slower utilization by tissues in the SHR: in spite of a lower starting level and smaller magnitude of insulin release, its level at the 60th min after glucose infusion in the SHR remains relatively higher, while in the normotensive Wistar rats the insulin level drops significantly (Fig. 3a).

A similar reduced rate of insulin utilization was observed earlier by Baumann et al. (1971) in patients with essential hypertension and attributed to a higher insulin "resistance" of the tissue of these patients. It may be suggested that the cause of this "resistance" has the same nature as the effects observed in spontaneously hypertensive rats and that it exerts an inhibiting action on the specific function of the insulin-producing tissue of the pancreatic islets.

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