

EXPERIMENTAL STUDY

The pancreatic islets in spontaneously hypertensive rats: islet blood flow and insulin production

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Abstract

The aim of the study was to investigate if hypertension affects pancreatic islet blood flow and endocrine function. For this purpose, spontaneously hypertensive rats (SHR) were compared with normotensive control Wistar–Kyoto rats (WKY). Both islet size and islet cell replication in 4-month-old SHR was increased compared with WKY. The (pro)insulin biosynthesis was reduced in islets isolated from SHR, whereas the insulin content was unchanged. A hyperinsulinemic response to glucose *in vivo* was observed in 4- and 12-month-old SHR. Pancreatic blood flow, measured using a microsphere technique, was lower in SHR than in WKY in rats aged 5 weeks, 4 months or 1 year. Islet blood flow was lower in 4-month-old and 1-year-old SHR. In 4-month-old animals, islet blood flow was unaffected by administration of enalaprilate and prazosin in both strains, but was markedly decreased by the administration of N^G-methyl-L-arginine. It was concluded that the islets of SHR have a decreased insulin production *in vitro* and a decreased islet blood perfusion. The reasons for this are likely to be multifactorial. Because SHR maintained an essentially normal glucose tolerance, an adaptation of the β -cells to the metabolic and hemodynamic changes imposed by hypertension occurred.

European Journal of Endocrinology 144 169–178

Introduction

It is well known that type 2 diabetes often occurs together with essential hypertension (1). Furthermore, recent epidemiological studies have shown that hypertension is a risk factor for the subsequent development of type 2 diabetes (2). The reason for this association could be that patients with essential hypertension are insulin resistant *per se*, without any relation to the degree of obesity (3). Because the ability of insulin to stimulate glucose uptake varies widely in normal individuals (4), it seems that type 2 diabetes might not develop as long as the pancreatic β -cells secrete sufficient quantities of insulin to maintain a normal glucose homeostasis (5). Therefore, a better understanding of the adaptation of β -cell function to metabolic aberrations in hypertension would provide a basis for the prevention of type 2 diabetes.

The spontaneously hypertensive rat (SHR) is one of the most widely used experimental models of essential hypertension. The clinical course is similar to that of human hypertension, even to the extent that hypertension-related functional and morphological abnormalities in both large and small vessels of different organs

develop with time (6). Furthermore, insulin resistance (7) and an increased β -cell sensitivity for glucose-stimulated insulin secretion (8) have been described in these animals (7), which is also in accordance with findings in patients with essential hypertension (3).

The increased vascular resistance in SHR is not associated with any marked changes in organ blood perfusion compared with control rats (9, 10). However, the blood flow responses to endothelium-derived vasoactive substances are enhanced with regard to vasoconstrictors (11), but attenuated with regard to the effects of the vasodilators (12, 13). Interestingly, the complex vasculature of the pancreatic islets (14) is exquisitely sensitive to locally produced endothelial factors (15–17), and may therefore be influenced by hypertension. An enhanced islet blood flow has been consistently observed in other rat models with an increased functional demand for insulin secretion (18–20). It is therefore conceivable that a high islet blood perfusion plays a crucial role in maintaining the β -cell hyperfunction required to compensate for insulin resistance in SHR.

Despite these conjectures, it is at present unknown whether islet blood flow is altered in SHR, and, if so,

whether this is of any functional significance. In the present study we first studied the age-related changes in islet blood flow in SHR and normotensive Wistar-Kyoto control rats (WKY) at a prehypertensive stage (5-week-old), during established hypertension (4-month-old), and finally during the stage when hypertensive organ damage has occurred (1-year-old). Results from previous studies suggest that the hypertension of SHR can be reduced by most conventional antihypertensive drugs. In view of this, we evaluated the acute effects of some such drugs, namely inhibitors of angiotensin-converting enzyme, nitric oxide formation and α_1 -adrenoceptors, all of which are known to also affect islet blood flow (16, 17, 21), on islet blood flow in 4-month-old SHR and WKY.

Because marked changes in hormone secretion (8, 22) as well as a diminished islet cell replication (23) have been described in 4-month-old SHR, we also investigated the function and growth of the β -cells in animals of this age.

Materials and methods

Animals

Male 4-week- and 4-month-old SHR and WKY were purchased from Møllegaard Breeding and Research Center A/S (Skensved, Denmark). The animals had free access to tap water and pelleted food throughout the experiments unless stated otherwise. Some of the animals were kept until 1 year old. All experiments were approved by the local animal ethics committee at Uppsala University.

Intraperitoneal glucose tolerance test

An intraperitoneal glucose tolerance test (ipGTT) was performed when the animals were 4 months or 1 year old. The animals (WKY $n = 7$, SHR $n = 8$ at 4 months; WKY $n = 11$, SHR $n = 10$ at 1 year) were injected intraperitoneally with a 30% (w/v) D-glucose solution (2 g glucose/kg body weight) after an overnight fast. Blood samples were taken from the cut tip of the tail immediately before and 10, 30, 60 and 120 min after glucose administration. Blood glucose concentrations were measured with test reagent strips (ExacTech[®]; Baxter Travenol, Deerfield, IL, USA). Serum immunoreactive insulin concentrations were measured with radioimmunoassay (Insulin RIA kit; Pharmacia-Upjohn Diagnostic, Uppsala, Sweden) with rat insulin standard (Novo Research Institute, Bagsvaerd, Denmark).

Blood flow measurements in rats of different ages

This procedure has been described in detail previously (24). Briefly, 5-week-old, 4-month-old or 1-year-old WKY or SHR were anesthetized with an intraperitoneal

injection of sodium pentobarbital (60 mg/kg body weight; Svenska Apoteksbolaget, Umeå, Sweden), heparinized and placed on an operating table maintained at body temperature. Polyethylene catheters were inserted into the ascending aorta, via the right carotid artery, and into the left femoral artery. The aortic catheter was connected to a pressure transducer (PDCR 75/1; Druck Ltd, Groby, UK). When the blood pressure had remained stable for at least 15 min, a total of $1.5\text{--}3.0 \times 10^5$ non-radioactive microspheres (NEN-Trac; DuPont Pharmaceuticals, Wilmington, DE, USA), with a diameter of 11 μm , were injected via the catheter with its tip in the ascending aorta over 10 s. Starting 5 s before the microsphere injection, and continuing for a total of 60 s, an arterial blood sample was collected by free flow from the catheter in the femoral artery at a rate of approximately 0.5 ml/min. The exact withdrawal rate was confirmed in each experiment by weighing the sample. Arterial blood was collected for determination of blood glucose and serum insulin concentrations as described above. The pancreas and adrenal glands were removed, blotted, weighed and treated with a freeze-thawing technique that visualized the pancreatic islets and microspheres (25). After the estimation of pancreatic islet volume (see below), the microspheres in the whole pancreas, islets, adrenal glands and arterial reference sample were counted and the blood flow values were calculated (24). Blood flow values based on the microsphere content of the adrenal glands were used to confirm that the microspheres were adequately mixed in the circulation. A difference of $<10\%$ in the blood flow values was taken to indicate sufficient mixing.

Blood flow measurements with non-radioactive, black microspheres after administration of enalaprilate, prazosin and N^G-monomethyl-L-arginine

These experiments were only performed in 4-month-old rats. The animals were prepared as described above. When the blood pressure had remained stable for 15 min, an intravenous injection of 0.4 ml of saline alone (control rats) or enalaprilate (25 $\mu\text{g}/\text{kg}$ body weight); Merck Sharp & Dohme BV, Haarlem, The Netherlands), prazosin (0.2 mg/kg body weight; Sigma Chemicals Co., St Louis, MO, USA) or N^G-monomethyl-L-arginine (L-NMMA; 10 mg/kg body weight; Calbiochem-Novabiochem, La Jolla, CA, USA) dissolved in saline were injected into the right femoral vein. Black microspheres (E-Z Trac[®]; IMT, Irvine, CA, USA) were then administered as given above 10 min after injection of one of the drugs. The organs were removed and the microsphere contents of the reference samples, whole pancreas, islets and adrenals were determined as described above. After counting the number of microspheres, the whole pancreas was digested with

sodium hydroxide (1 M) and sonicated (26). The number of microspheres in the digested pancreas was determined by counting samples transferred to glass microfiber filters. The digestion method correlated well with the direct counting method ($n = 25$, $r = 0.98$; $P < 0.0001$).

Estimation of pancreatic islet volume

This procedure has previously been described in detail (27). Briefly, the whole pancreas was cut into small pieces weighing approximately 20 mg each. After visualization of the islets with the freeze-thawing technique, the volume of the islets was determined by a point-counting method (28) by an observer unaware of the origin of the samples. Only islets with a diameter exceeding 50 μm were counted. The islet volume was used to express islet blood flow per islet mass by multiplying the pancreatic weight with the mean islet volume fraction of whole pancreas in each strain.

Chemicals

The chemicals were as follows: collagenase prepared from *Clostridium histolyticum* (Boehringer-Mannheim, Mannheim, Germany); culture medium RPMI 1640 (11.1 mmol/l glucose) and fetal calf serum (FCS) (HyClone, Cramlington, UK); bovine serum albumin (BSA) (Miles, Slough, UK); L-[4,5- ^3H]leucine, D-[U- ^{14}C]glucose, [methyl- ^3H]thymidine (Amersham International, Amersham, Bucks, UK); Hepes and antimycin A (Sigma Chemical Co.); Hanks' solution (SBL Vaccin AB, Stockholm, Sweden); emulsion Kodak NTB-2, developer Kodak D-19, fixative F-24 (Kodak (Sollentuna, Sweden); hyamine hydroxide (ICN Immunobiologicals, Lisle, IL, USA and New England Nuclear, Boston, MA, USA).

Islet isolation and culture

In each experiment pancreatic islets isolated from one SHR and one WKY by using a collagenase digestion procedure (29) were studied in parallel. Islets were studied immediately (day 0) or after 2 days of culture (day 2). For this purpose, groups of 150 islets were cultured free-floating for 2 days at 37 °C in an atmosphere of humidified air + 5% CO_2 in 4.5 ml of RPMI 1640 supplemented with 0.5 ml FCS.

Measurement of DNA synthesis

Duplicate groups of 25 islets (day 2) were incubated with 1 $\mu\text{Ci/ml}$ [methyl- ^3H]thymidine at 11.1 or 28.0 mmol/l glucose for 16 h in RPMI 1640 + 10% FCS. The islets were then rinsed in Hanks' solution containing 10 mmol/l thymidine and sonicated in 250 μl redistilled water. The homogenate was precipitated in ice-cold 10% trichloroacetic acid.

Duplicate samples of the homogenate were analyzed for DNA content (30).

Autoradiographic estimates of islet DNA synthesis were performed as follows. From the same incubations used for the DNA synthesis experiments, groups of 75 islets were collected, washed and fixed. Histological slides were dipped in Kodak NTB-2 emulsion, exposed for 8 weeks at 4 °C, and developed in Kodak D-19 at 20 °C for 6 min, fixed in Kodak F-24 for 10 min and finally stained with hematoxylin. To determine the islet cell labeling index, radioactively labeled nuclei were counted at a magnification of $\times 1000$ by an observer unaware of the origin of the slides, and expressed as a percentage of the total number of nuclei scored. A labeled cell was defined as containing at least 10 grains over the nucleus.

Glucose-stimulated insulin release and islet content of insulin and DNA

Groups of 10 islets (day 0) were transferred in triplicate to sealed glass vials containing 0.25 ml of Krebs-Ringer bicarbonate buffer supplemented with 10 mmol/l HEPES (KRBH), and 2 mg/ml BSA. The islets were incubated at 37 °C in a gas phase of 95% O_2 + 5% CO_2 with 1.67 mmol/l glucose for 1 h, and then replaced by KRBH supplemented with 16.7 mmol/l glucose for an additional hour. The islets were harvested after the incubations, pooled in groups of 30 and homogenized in 0.2 ml redistilled water. A fraction of the homogenate was mixed with acid ethanol, and insulin was extracted overnight at 4 °C. The insulin concentration of the extract was measured by radioimmunoassay (31). Another fraction of the aqueous homogenate was used for DNA measurements (30).

Islet glucose oxidation rate

Groups of 10 islets each (day 0) were incubated in triplicate in 100 μl KRBH without BSA supplemented with D-[U- ^{14}C]glucose and non-radioactive glucose and measured as described previously (32).

Islet (pro)insulin and total protein biosynthesis rates

Duplicate groups of 10 islets (day 0) were incubated for 2 h at 37 °C (air + 5% CO_2) in 100 μl KRBH supplemented with 2 mg/ml BSA and 50 $\mu\text{Ci/ml}$ L-[4,5- ^3H]leucine and 16.7 mmol/l glucose and homogenized in water. The total protein biosynthesis rate was measured after trichloroacetic acid precipitation of a fraction of the homogenate. The (pro)insulin biosynthesis rate was determined by an immunabsorption technique (33).

Measurement of insulin mRNA

Groups of 50 islets (day 0) were washed in phosphate-buffered saline and briefly sonicated in 200 μ l of 0.1 mol/l Tris (pH 7.5), 10 mmol/l EDTA and 1% sodium dodecyl sulphate (SDS). The sonicates were extracted with 200 μ l of phenol/chloroform/isoamyl-alcohol. Nucleic acids were precipitated and electrophoresed on 1% agarose gels containing formaldehyde and blotted to GeneScreen membranes. After baking, the membranes were hybridized to [32 P]dCTP labeled rat insulin I cDNA (34). After washing, the membranes were exposed to Hyperfilm MP for autoradiography and densitometry.

Morphometry of cultured islets

In preliminary experiments a decrease in the DNA content of SHR islets was noted after culture (data not shown). To further investigate this, measurements of possible necrotic islet areas were performed on day 2 of culture in separate experiments. Groups of 75 islets were fixed, embedded, sectioned and stained with hematoxylin and eosin. The total islet area and the central necrotic area were determined by using computerized morphometry (MOP-Videoplan; Zeiss Svenska AB, Stockholm, Sweden). The average number of islets examined (i.e. islets derived from one animal) was 41 ± 7 for WKY and 44 ± 5 for SHR respectively. Necrotic areas were defined as homogeneously dense, eosinophilic areas, sometimes containing pyknotic nuclei. The percentage of the necrotic area per islet was calculated by dividing the total necrotic areas by total islet area.

Statistical analysis

All values are given as means \pm S.E.M. When two groups were compared, Student's two-tailed, unpaired *t*-test was used. When multiple comparisons were made, the data were compared by one-way or two-way repeated measures analysis of variance (ANOVA) followed by Fisher's protected least statistical difference test using StatView[®] (Abacus Concepts, Berkeley, CA, USA). The results were considered to be significant when $P < 0.05$.

Results

General characteristics of animals of different ages

Body weight did not differ between WKY and SHR at any age. Mean arterial blood pressure was significantly higher in SHR at all ages. The blood pressure in 4-month and 1-year-old SHR was higher than in 5-week-old SHR ($P < 0.01$ for both comparisons), whereas in WKY the blood pressure was similar in all age groups.

Table 1 Characteristics of Wistar–Kyoto (WKY) or spontaneously hypertensive rats (SHR) at 5 weeks, 4 months and 1 year of age.

	5 weeks	4 months	1 year
Number of animals			
WKY	12	11	9
SHR	8	10	7
Body weight (g)			
WKY	62 \pm 3	330 \pm 7	440 \pm 5
SHR	68 \pm 3	332 \pm 6	439 \pm 5
Blood glucose (mmol/l)			
WKY	4.9 \pm 0.3	3.9 \pm 0.1	4.0 \pm 0.1
SHR	5.1 \pm 1.3	4.6 \pm 0.2*	4.9 \pm 0.2*
Serum insulin concentration (μ U/ml)			
WKY	40 \pm 6	61 \pm 10	198 \pm 17
SHR	56 \pm 9	68 \pm 10	163 \pm 17
Mean arterial blood pressure (mmHg)			
WKY	82 \pm 2	88 \pm 3	68 \pm 4
SHR	113 \pm 15*	151 \pm 12**	149 \pm 13**
Heart weight (g)			
WKY	0.27 \pm 0.01	0.94 \pm 0.02	1.18 \pm 0.02
SHR	0.31 \pm 0.01	1.13 \pm 0.04**	1.60 \pm 0.04**
Pancreatic weight (g)			
WKY	0.30 \pm 0.01	1.04 \pm 0.02	1.24 \pm 0.03
SHR	0.34 \pm 0.01	1.00 \pm 0.03	1.35 \pm 0.04*
Islet volume of whole pancreas (%)			
WKY	1.47 \pm 0.14	1.90 \pm 0.08	2.42 \pm 0.21
SHR	1.43 \pm 0.17	2.65 \pm 0.21**	4.02 \pm 0.23**

Values are means \pm S.E.M. * $P < 0.05$ and ** $P < 0.001$ vs. WKY of the same age by ANOVA.

The heart weight was increased in SHR compared with WKY at 4 months and 1 year of age. The pancreatic weight was increased slightly in SHR at 1 year of age compared with that of WKY (Table 1).

Blood glucose and serum insulin concentrations in animals of different ages

The blood glucose concentrations at the time of the islet blood flow measurements were slightly, but significantly, higher in SHR than WKY at 4 months and 1 year of age (Table 1). Serum insulin concentrations did not differ between the strains, but was increased at 1 year of age when compared with that of the younger age groups of the same strain ($P < 0.001$; Table 1). There were no strain differences in the blood glucose or serum insulin concentrations during an ipGTT at either 4 months or 1 year of age (Fig. 1). However, the area under curve (AUC) for glucose was decreased in SHR compared with WKY at 4 months of age, whereas the AUC for insulin did not differ (Table 2). The ratio of AUC for insulin to glucose was higher in SHR than in WKY at this age. In 1-year-old animals, AUC for glucose did not differ between WKY and SHR, whereas the AUC for insulin was increased in SHR. Thus, the ratio of AUC for insulin to glucose was higher in SHR than in WKY also at 1 year of age. The AUC for glucose and insulin, and the ratio, were increased in 1-year-old rats when compared with those aged 4 months in both strains.

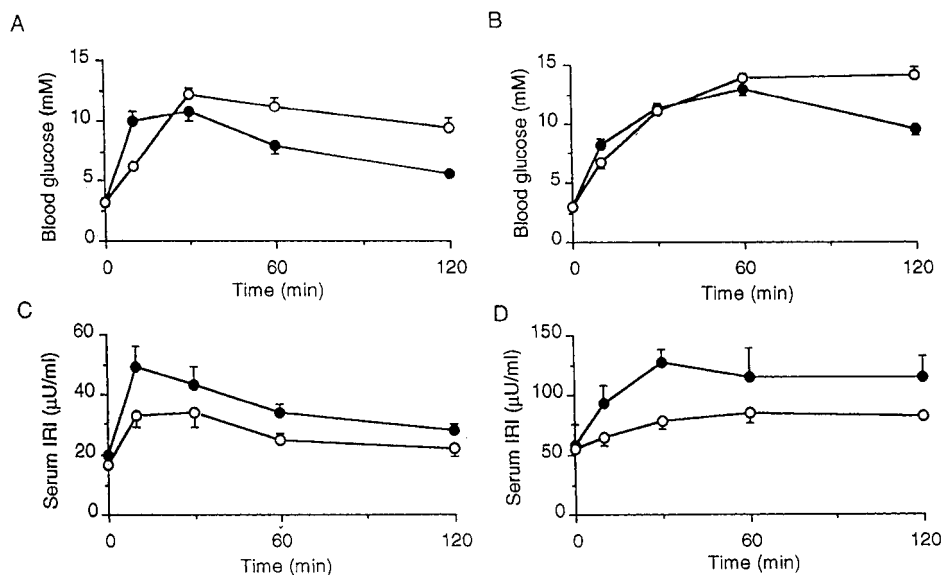


Figure 1 Blood glucose (A, B) and serum insulin concentrations (C, D) during an intraperitoneal glucose tolerance test (2 g/kg body weight), after an overnight fast, at 4 months of age (A, C) and 1 year of age (B, D). WKY (● $n = 7$ at 4 months, $n = 11$ at 1 year) and SHR (○; $n = 8$ at 4 months, $n = 10$ at 1 year). Values are means \pm S.E.M.

Pancreatic islet volume in animals of different ages

The islet volume did not differ between WKY and SHR at 5 weeks of age, but was higher in SHR at both 4 months and 1 year of age. The fractional islet volume increased with age in both strains (5 weeks vs 4 months: $P < 0.05$ in WKY, $P < 0.001$ in SHR; 4 months vs 1 year: $P < 0.05$ in WKY, $P < 0.001$ in SHR; all comparisons made with ANOVA).

Blood flow measurements in animals of different ages

There were no differences in whole pancreatic blood flow between WKY and SHR in any of the age groups studied. However, pancreatic blood flow decreased with age in both strains. Islet blood flow, corrected for estimated islet weight, was similar in WKY and SHR at 5 weeks of age, whereas it was decreased in SHR compared with WKY at 4 months and 1 year of age. In

both strains, islet blood flow was higher in 4-month-old than 5-week-old animals. No further increase in islet blood perfusion from 4 months to 1 year of age was observed in either strain. Fractional islet blood flow was very low at 5 weeks of age in both WKY and SHR, and increased with age in both strains. The fractional islet blood flow was higher in SHR than in WKY at both 4 months and 1 year of age (Table 3).

Effects of enalaprilate, prazosin and L-NMMA on 4-month-old rats

The body weights were similar in all these animals (data not shown), whereas the pancreatic islet volume was almost doubled in the SHR compared with WKY (2.85 ± 0.21 vs. $1.59 \pm 0.18\%$; $P < 0.001$).

The blood glucose and serum insulin concentrations were similar in saline-treated SHR and WKY at the time of the blood flow measurements. Enalaprilate slightly increased blood glucose concentrations, whereas the other substances had no such effects (Table 4).

Table 2 Area under the curve (AUC) during an intraperitoneal glucose tolerance test in Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) at 4 months and 1 year of age.

	4 months		1 year	
	WKY	SHR	WKY	SHR
Glucose (mmol \times min)	1196 \pm 75	957 \pm 60*	1514 \pm 54§	1307 \pm 121§§
Insulin concentrations (μ U/ml \times min)	3212 \pm 279	4252 \pm 422	9482 \pm 582§§§	13505 \pm 2135***§§§
Ratio of AUC insulin:glucose	2.8 \pm 0.4	4.4 \pm 0.3*	6.3 \pm 0.5§§§	10.2 \pm 0.9***§§§

Values are means \pm S.E.M. for 8 (4 months) or 10 (1 year) observations. * $P < 0.05$. ** $P < 0.01$ and *** $P < 0.001$ vs. WKY. § $P < 0.01$ and §§ $P < 0.001$ vs. 4-month-old animals of the same strain. All comparisons were made with ANOVA.

Table 3 Pancreatic and islet blood flow values in Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) at 5 weeks, 4 months and 1 year of age.

	Strain	Age		
		5 weeks	4 months	1 year
Pancreatic blood flow (ml/min × g)	WKY	1.47 ± 0.25	0.87 ± 0.07§§	0.91 ± 0.10§§
	SHR	1.07 ± 0.10	0.47 ± 0.07§	0.57 ± 0.09§
Islet blood flow (μl/min × mg estimated islet mass)	WKY	1.8 ± 0.4	4.9 ± 0.4§§§	4.6 ± 0.06§§§
	SHR	1.5 ± 0.4	2.3 ± 0.2**	2.2 ± 0.3**
Fractional islet blood flow (% of pancreatic blood flow)	WKY	1.6 ± 0.2	10.7 ± 0.4§§§	12.4 ± 0.9§§§§
	SHR	1.9 ± 0.5	13.4 ± 0.7**§§§	15.3 ± 0.7**§§§§

Values are means ± S.E.M. for the number of observations given in Table 1. * $P < 0.01$ and ** $P < 0.001$ vs. WKY. § $P < 0.05$, §§ $P < 0.01$ and §§§ $P < 0.001$ vs. 5-week-old animals of the same strain. \$ $P < 0.05$ vs. 4-month-old animals of the same strain. All comparisons were made with ANOVA.

Enalaprilate decreased serum insulin concentrations in both WKY and SHR, whereas prazosin decreased insulin concentrations only in SHR. L-NMMA decreased serum insulin concentrations in WKY, but had no effects in SHR.

Basal mean arterial blood pressure was higher in SHR than in WKY before administration of any of the test substances and at all time points in the saline-treated rats (Table 5). Enalaprilate lowered blood pressure 3 min after administration compared with corresponding saline-treated animals (60 ± 2 mmHg, $P < 0.001$ in WKY and 120 ± 8 mmHg, $P < 0.01$ in SHR), but after 5 and 10 min it did not differ from the corresponding control rats. In WKY, prazosin caused a slight reduction in blood pressure after 5 min but 10 min after administration no effects were seen. In SHR, however, prazosin markedly decreased blood pressure during the 10-min observation period. L-NMMA increased blood pressure in WKY both 5 and 10 min after administration. In SHR L-NMMA increased blood pressure only 5 min after injection.

Whole pancreatic blood flow was lower in SHR than WKY in the saline-injected group and in rats administered L-NMMA (Table 4). Enalaprilate, prazosin and L-NMMA

decreased pancreatic blood flow in WKY, but not in the SHR compared with the corresponding saline-injected animals. Islet blood flow was lower in SHR than in WKY in animals administered saline, prazosin or L-NMMA. Islet blood flow was not affected by enalaprilate or prazosin in WKY or SHR compared with the corresponding saline-injected rats of the same strain. L-NMMA decreased islet blood flow in WKY and SHR. When islet blood flow was corrected for differences in islet volume between the strains, the blood perfusion was markedly decreased in all groups of SHR compared with WKY.

In vitro studies of pancreatic islets from 4-month-old rats

The DNA synthesis rate *in vitro* was increased in SHR islets when compared with WKY islets after both stimulation with 11.1 mM (1667 ± 133 vs. 1116 ± 116 d.p.m./islet × 16 h × μg DNA; $n = 6$, $P < 0.05$) and 28.0 mM (1394 ± 111 vs. 1061 ± 137 d.p.m./islet × 16 h × μg DNA; $n = 6$, $P < 0.05$) glucose. Islet cell labeling index was increased in SHR islets at

Table 4 Blood glucose concentrations, serum insulin concentrations, pancreatic (PBF) and islet blood flow values in 4-month-old anesthetized Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) 10 min after an intravenous injection of 0.4 ml of enalaprilate (25 μg/kg body weight), prazosin (0.2 mg/kg body weight), N^G-nitro-L-arginine (10 mg/kg body weight) dissolved in saline or saline alone.

	Blood glucose (mmol/l)	Serum insulin (μU/ml)	Pancreatic blood flow (ml/min × g)	Islet blood flow (μl/min × mg islets)	% of PBF
Saline					
WKY (7)	3.9 ± 0.2	147 ± 17	1.57 ± 0.27	8.43 ± 0.82	11.0 ± 0.9
SHR (6)	4.4 ± 0.2	164 ± 11	0.71 ± 0.13§§	3.16 ± 0.46§§§	12.0 ± 0.6
Enalaprilate					
WKY (8)	5.0 ± 0.4**	50 ± 7***	0.98 ± 0.10*	6.42 ± 0.85	12.5 ± 1.1
SHR (7)	5.0 ± 0.2	58 ± 5***	0.52 ± 0.11	2.96 ± 0.50§§	17.1 ± 1.9**§§
Prazosin					
WKY (7)	4.0 ± 0.1	127 ± 17	0.92 ± 0.17*	6.37 ± 1.63	12.4 ± 1.3
SHR (5)	4.2 ± 0.3	264 ± 18***§§§	0.47 ± 0.11	2.05 ± 0.42§§§	13.0 ± 2.1
N ^G -nitro-L-arginine					
WKY (7)	3.5 ± 0.1	63 ± 8***	1.06 ± 0.30*	4.53 ± 0.95**	9.0 ± 0.6
SHR (7)	4.0 ± 0.3	137 ± 18§§§	0.42 ± 0.11§	0.96 ± 0.23*§§	6.7 ± 1.0**

Values are means ± S.E.M. for the number of experiments given within parenthesis. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ when compared with saline-injected animals of the same strain. § $P < 0.05$, §§ $P < 0.01$ and §§§ $P < 0.001$ when compared with WKY receiving the same treatment. All comparisons were made with ANOVA.

Table 5 Mean arterial blood pressure in anesthetized Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) before and 5 and 10 min after an intravenous injection of 0.4 ml of enalaprilate (25 µg/kg body weight), prazosin (0.2 mg/kg body weight), N^G-nitro-L-arginine (10 mg/kg body weight dissolved in saline or saline alone).

Time point (min)	0	5	10
Saline			
WKY (7)	73 ± 3	74 ± 2	73 ± 3
SHR (6)	146 ± 6§§§§	146 ± 7§§§§	138 ± 7§§§§
Enalaprilate			
WKY (8)	76 ± 3	61 ± 2**	81 ± 6
SHR (7)	143 ± 10***	135 ± 9§§§§	158 ± 8§§§§
Prazosin			
WKY (7)	66 ± 3	59 ± 2*	63 ± 2
SHR (5)	163 ± 4§§§§	85 ± 2***§§	82 ± 3***§
N ^G -nitro-L-arginine			
WKY (7)	74 ± 4	97 ± 3**	92 ± 4*
SHR (7)	134 ± 9§§§§	170 ± 7**§§§§	139 ± 11§§§§

Values are means ± S.E.M. for the number of experiments given within parenthesis. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with the value at time in rats of the same strain receiving the same treatment. § $P < 0.05$, §§ $P < 0.01$ and §§§ $P < 0.001$ compared with the corresponding value in WKY receiving the same treatment.

28.0 mM glucose (4.1 ± 1.1 vs. $1.0 \pm 0.2\%$; $n = 6$, $P < 0.05$), but not at 11.1 mM (2.7 ± 0.8 vs. $1.5 \pm 0.6\%$; $n = 6$) glucose. The fraction of the central necrotic area to total islet area observed on day 2 of culture was markedly higher in SHR than in WKY ($22.9 \pm 2.6\%$ in SHR vs $11.2 \pm 2.8\%$ in WKY; $P < 0.05$, $n = 6$).

Freshly isolated islets had a higher DNA content in SHR than in WKY (Table 6). The insulin release after stimulation with 1.67 mM glucose and the insulin content were also increased in SHR, but when these values were expressed per DNA content no differences were seen. Insulin release after stimulation with 16.7 mM glucose did not differ between WKY and SHR. No differences in islet glucose oxidation rates between the two groups of animals were seen.

The rates of (pro)insulin biosynthesis and the ratio between (pro)insulin biosynthesis and total protein

biosynthesis were decreased in SHR, whereas the total protein biosynthesis rate was similar in SHR and WKY (Table 6). The insulin mRNA content appeared to be higher in WKY compared with SHR islets, although the difference was not statistically significant ($0.05 < P < 0.10$).

Discussion

The occurrence of insulin resistance and compensatory hyperinsulinemia has been consistently reported in SHR (7, 35). In the present study, no change in basal serum insulin concentrations was seen, but the increased ratio of the AUC for serum insulin to blood glucose concentrations confirmed the presence of a mild insulin resistance. It was recently demonstrated that insulin secretion was enhanced from the isolated pancreas of SHR in response to an increase in ambient glucose concentration from 5 to 10 mmol/l, i.e. a subnormal glucose set-point (ED_{50}) for insulin secretion (8, 22). Chen and co-workers (8) showed that the glucokinase activity in SHR islets was increased by 40% compared with WKY. They therefore proposed that an increased β -cell sensitivity for glucose, namely, an exaggerated insulin output at a normal glucose concentration, was a major sign of β -cell adaptation to insulin resistance in SHR. Furthermore, it was suggested that β -cell glucokinase activity played a key role in regulating the set-point for glucose-induced insulin secretion. In the present study we observed an enhanced insulin release *in vitro* at a glucose concentration of 1.67 mmol/l from isolated SHR islets. However, we cannot exclude that this could be attributed to the increased islet size.

For the *in vitro* experiments and the evaluations of blood flow regulation, 4-month-old rats were chosen, because the vascular changes and increases in islet volume were fully manifested at this time point in SHR. When expressed per islet, the rate of (pro)insulin biosynthesis in the SHR exhibited a decrease of approximately 30% but this was not sufficient to affect

Table 6 DNA content, insulin secretion, insulin content, glucose oxidation rate, (pro)insulin and total protein biosynthesis rates and insulin mRNA content in islets isolated from Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR).

	WKY	SHR
DNA content (µg/10 islets)	0.52 ± 0.04	0.81 ± 0.08**
Insulin release (pmol/10 islets × 60 min)		
1.67 mmol/l glucose	0.6 ± 0.1	0.9 ± 0.2*
16.7 mmol/l glucose	9.3 ± 1.5	7.9 ± 1.2
Insulin content (pmol/10 islets)	98 ± 7	13.0 ± 9**
(pmol/µg DNA)	200 ± 26	183 ± 38
Glucose oxidation rate (pmol/10 islets × 90 min)	604 ± 92	788 ± 128
(Pro)insulin biosynthesis rate (kdpm/10 islets × 2 h)	21.7 ± 3.2	14.8 ± 2.3*
Total protein biosynthesis rate (kdpm/10 islets × 2 h)	89.3 ± 13.6	95.3 ± 16.4
Fraction (pro)insulin of total protein biosynthesis (%)	26.0 ± 3.8	17.0 ± 2.3**
Insulin mRNA content (optical density/50 islets)	1.32 ± 0.31 (6)	0.49 ± 0.20 (4)

Values are means ± S.E.M. for 7 observations unless otherwise indicated. * $P < 0.05$ and ** $P < 0.01$ vs. WKY by Student's paired t-test except the last line where unpaired t-test was used.

either the islet insulin content or insulin secretory capacity. A difference in (pro)insulin biosynthesis rate and unchanged insulin content might indicate a difference in the intracellular degradation of insulin (33). In another study there was an increased islet insulin content associated with an unchanged rate of (pro)insulin biosynthesis and intracellular insulin degradation in SHR (22). It should be noted that the insulin content in the latter study is given per islet, and not corrected for DNA content. Our findings may therefore reflect the size difference between the islets isolated from WKY and SHR. The reasons for the differences with regard to (pro)insulin biosynthesis may be explained by genome differences or by differences in experimental procedures.

It has previously been reported that the β -cell fraction per islet did not differ between WKY and SHR (36). A decreased insulin production in SHR could in this case be compensated for by an increased islet size to meet the insulin demand. However, the larger SHR islets are likely to be more exposed to hypoxia during *in vitro* culture as a result of diffusion problems. This idea was confirmed by the increased degree of central necrosis observed in SHR islets after 2 days of culture. For this reason, all *in vitro* evaluations of islet functions were performed in freshly isolated islets.

The islet volume per pancreas was significantly increased in SHR at 4 months and at 1 year of age. Furthermore, the DNA content of freshly isolated islets was significantly increased in SHR. This could reflect a compensatory β -cell growth in response to insulin resistance in these animals (37), as discussed below. In addition, it should be noted that in rats the islet mass increases throughout postnatal life (38). In the present study, islet cell replication as estimated by the incorporation of tritiated thymidine was significantly higher in SHR than in WKY. It was previously suggested that the rate of β -cell regeneration was lower in SHR compared with WKY, as SHR became more hyperglycemic than WKY after a 90% pancreatectomy (39) or neonatal streptozotocin injections (40). This difference may be explained by a suppressive effect of hyperglycemia on the replication activity *in vivo* in SHR, or that there is a genetically predetermined maximal level for this capacity in SHR.

To what extent a hypoperfusion of the pancreatic islets, as seen in the SHR in the present study, affects their function *in vivo* is largely unknown. However, the SHR investigated in this study did not develop glucose intolerance, despite an almost 50% reduction in islet blood flow compared with age-matched WKY, and despite the slight decrease in insulin production observed in the *in vitro* studies. These findings underline the versatility and large margin of reserve in islet blood flow and other compensatory β -cell mechanisms for the maintenance of a normal carbohydrate homeostasis. It is noteworthy that, as mentioned above, SHR become more hyperglycemic than WKY when their islet mass is

reduced (39, 40, 41). To what extent this is because of insulin resistance in the SHR, or to the combined effects of an insufficient blood perfusion and a decreased number of β -cells is at present unknown.

The islet blood flow was fairly low at 5 weeks of age compared with the older age groups, thereby confirming a previous report (42). It should be noted that younger animals have smaller islets and a smaller total islet volume (cf. 38). Because the vasculature of the smaller islets is in continuity with the exocrine capillary system (14), the blood perfusion in these islets is likely to be regulated by mechanisms common to those of the exocrine pancreas (15). Support for this idea comes from the findings of a fractional islet blood flow similar to that of the volume contributions of the islets in this age group. An alternative explanation is that the islets of younger animals contain an immature vascular system that has not as yet obtained blood flow regulatory mechanisms characteristic of adults.

To compensate for the changes in islet volumes between 4-month-old and 1-year-old SHR and WKY we chose to express islet blood flow as per estimated islet mass. This mode of expression showed a markedly lower rate of islet blood flow in both these age groups of SHR compared with age-matched WKY. The present findings suggest that, expressed on this basis, islet blood flow was constant between 4 and 12 months of age. Despite the reduction in islet blood perfusion in SHR a glucose load was associated with a slightly hyperinsulinemic response, as evidenced by the increased AUC for the insulin response and increased basal insulin secretion of SHR islets *in vitro*. This is in line with previous observations of a β -cell hyperfunction in SHR (7, 8). It should be noted that an enhanced islet blood perfusion has been consistently observed in normoglycemic and hyperglycemic rat models with an increased functional load on the pancreatic β -cells (16, 18–20). Because the blood supply is of crucial importance, not only for the metabolism of the islets, but also to facilitate the delivery of islet hormones to their target organs, the findings in these previous studies strongly support the view that islet blood flow usually accommodates to the need for insulin secretion. In contrast to this, even though the SHR have some degree of insulin resistance, these animals had a decreased islet blood perfusion during manifested hypertension compared with the normotensive control rats. It should be noted that the islet blood perfusion in the obese and insulin-resistant *ob/ob* mouse shows a similar pattern, but in these animals the islet blood perfusion changes are likely to depend on the prevailing marked hyperglycemia in this animal model (27), i.e. a condition not present in SHR rats. Another factor of importance when interpreting the findings in SHR is that the islet blood flow is decreased in concert with the whole pancreatic blood flow, whereas the effects on blood flow in the other animal models are much more pronounced in the islets than in the exocrine tissue.

This suggests that different mechanisms are operating in the various animal models.

In order to further elucidate this topic we evaluated the influence of nitric oxide, angiotensin II and blockade of α_1 -adrenoceptors on islet blood perfusion in 4-month-old SHR and WKY. In these studies we used another type of polystyrene microspheres that can be color-labeled (cf. 26). The differences between the flow values obtained with these microspheres and the standard Nen-Trac[®] microspheres were small, and were most probably due to the infusion of the extra volumes of saline. This has also been confirmed in further evaluations (unpublished observations).

Previous studies have demonstrated that an increased vascular resistance in the mesenteric circulation could contribute to the development of hypertension in SHR (43). In line with this is the suggestion that SHR have a different flow regulation in the mesenteric vascular bed caused by an enhanced myogenic response, which contributes to the early stages of hypertension (43). A third report shows that the number of small arterioles, i.e. resistance vessels, is reduced in mesenteric microvasculature compared with WKY (44). In confirmation of this we found a reduction in both whole pancreatic and islet blood flow. However, no regional differences in the intra-pancreatic blood flow between branches of the celiac and superior mesenteric artery were found (data not shown). The reasons for the increased vascular resistance in SHR are unknown, but it has been suggested that there is an imbalance between the production between endothelium-derived relaxing and contracting factors (45). Our present findings demonstrated no clear cut effects by inhibiting angiotensin-converting enzymes with enalaprilate. This contrasts to our previous findings in Sprague-Dawley rats where we noted a preferential sensitivity of the islet blood perfusion to angiotensin II (17). The present findings, however, suggest that angiotensin II is not primarily responsible for the islet blood flow changes seen in SHR. Blockade of α_1 -adrenoreceptors with prazosin did not preferentially affect islet blood flow in the SHR. Previous experiments have demonstrated that the islet vasculature responds to epinephrine with vasoconstriction (15), but there is some controversy with regard to the receptors involved (cf. 15, 46). It should also be noted in this context that plasma catecholamines in SHR and WKY of different ages do not differ, but the increment seen after stress is greater in SHR (10). In view of our findings, catecholamines also seem to be less likely candidates for the observed changes in SHR islet blood flow.

In contrast to the other tested substances, administration of L-NMMA, an inhibitor of mainly the constitutive form of nitric oxide synthase, led to a further decrease in islet blood flow in both strains, but the response was more marked in SHR. Previous experiments have demonstrated that the islet vasculature contains large amounts of the enzyme (47), and

is dependent on an undisturbed nitric oxide formation to maintain its normal high blood perfusion (16). Nitroprusside, a nitric oxide donor, dilates mesenteric resistance vessels to the same degree in SHR and WKY (12). However, it has been suggested that hypertension impairs endothelium-derived dilator responses, including acetylcholine (12, 13), due to a changed ratio of vasoconstrictors to vasodilators (cf. 13). Our present findings therefore confirmed the sensitivity of the islet vasculature to nitric oxide, but provided no evidence for any causative role of this substance in the islet blood flow changes seen in SHR. In summary, it seems as if neither increased activities in the adrenergic nervous system nor the angiotensin II system is involved in the decreased islet blood perfusion seen in SHR. It is also unlikely that nitric oxide *per se* is involved, although a decreased sensitivity of the vasculature to this substance cannot be ruled out. It therefore seems likely that there is multifactorial etiology for the islet hypoperfusion seen in SHR.

Acknowledgements

The skilled technical assistance of Birgitta Bodin, Eva Forsbeck, Astrid Nordin and Eva Törnelli is gratefully acknowledged. We also thank Nils Welsh for his help with the insulin mRNA measurements. Masanori Iwase was a visiting scientist from Kyushu University, Fukuoka, Japan, in a scientific exchange program between the Royal Swedish Academy of Sciences and the Japan Society for the Promotion of Science. Financial support was received from the Swedish Medical Research Council (72X-109, 12X-8273), the Swedish Diabetes Association, the Scandinavia-Japan Sasakawa Foundation, the Swedish-American Diabetes Research Program funded by the Juvenile Diabetes Foundation and the Wallenberg Foundation, the NOVO Nordic Fund, the Åke Wiberg Foundation and the Family Ernfors Fund.

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Received 21 March 2000

Accepted 11 October 2000