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Altered hyperemic response of the coronary arterial bed in alloxan-diabetes

M. Zs. Koltai, M. Wagner and G. Pogátsa[1]

Research Unit, National Institute of Cardiology, P.O.B. 9-88, H-1450 Budapest (Hungary), May 3, 1982

Summary. Reactive hyperemic responses of the coronary arterial bed, provoked by asphyxia or clamping of the coronary artery, were compared in alloxan-diabetic and metabolically healthy dogs. In alloxan-diabetic dogs the response of the coronary arterial bed lasted longer, and its reactivity to hypoxia was lower. Treatment with adenosine caused less vasodilation in diabetic animals than in controls. These changes may be due to the altered reactivity of diabetic vascular smooth muscle.

Ischemic heart diseases[2,3] and cerebrovascular alterations[4-6] are more frequent and severe in cases where diabetes mellitus is present. Biochemical and morphological changes and altered innervation of the coronary arteries may all play an important role in ischemia of the myocardium in diabetes. It has already been demonstrated that the reactivity of the coronary arterial bed to norepinephrine and to electrical stimulation of the cardiac plexus is considerably diminished in animals with diabetes as compared to metabolically healthy animals. These alterations have been detected even in early stages of diabetes when macro- or microangiopathy are not observed[7]. In the present study the reactivity of the coronary arterial bed to hypoxia was investigated during hyperemia provoked in different ways in alloxan-diabetic dogs.

Methods. 26 healthy young mongrel dogs of both sexes, each weighing 14–35 kg, were selected for the study. All received the same diet, consisting of 25% protein, 60% carbohydrate, 15% fat, vitamins and mineral salts ad libitum. 12 dogs were made diabetic using alloxan (560 mmoles/kg i.v., alloxan tetrahydrate, Merck). 14 dogs served as controls. Plasma disappearance rates of glucose[8], plasma glucose[9] and urea nitrogen[10] from venous blood were determined at the beginning of the study and at least once monthly during the observation period, as well as on the day before the hemodynamic investigation, in the fasting state. The glucose[9] and aceton[11] contents of urine collected over 24 h were determined at least once weekly during the observation period.

The hemodynamic investigation was carried out 3 months after the induction of diabetes. Blood flow in the left anterior descending coronary artery was measured continuously by an electromagnetic flowmeter (Godard-Statham, SP 2202) in dogs ventilated with positive pressure under

Metabolic and hemodynamic variables of control and alloxan-diabetic dogs

	Plasma disappearance rate of glucose (μmoles/min)	Plasma glucose (mmoles/l)	Glucose excretion (mmoles/day)	Body weight (kg)	Mean arterial blood pressure (kPa)	Coronary blood flow (ml/min)
Control (n = 14)	18 ± 1	4.96 ± 0.18	0 ± 0	22.1 ± 1.7	13.7 ± 0.7	38.2 ± 11.5
Diabetic animals before alloxan	18 ± 1	5.19 ± 0.23	0 ± 0	21.8 ± 1.0	-	-
After alloxan (n = 12)	7 ± 2[b]	14.35 ± 0.85[b]	496 ± 120[b]	18.8 ± 1.1[b]	17.5 ± 1.7[a]	52.2 ± 9.7

Data are expressed as mean ± SEM. [a]$p < 0.05$; [b]$p < 0.01$.

pentobarbital anesthesia (133 mmoles/kg, Nembutal, Serva). Mean arterial blood pressure was recorded in the thoracic aorta with a Statham gauge (p23Db) on a multiscriptor (Hellige 9400/6). The characteristics of reactive hyperemic responses, induced either by asphyxia lasting for 1 min (produced by switching off the ventilation) or by clamping of the left anterior descending coronary artery for 1 min, were determined in all animals. Thereafter, in 6 metabolically healthy and 6 alloxan-diabetic dogs the first side branch of the coronary artery was cannulated and after the registration of the basal values, 30, 60, 120, 240 or 480 nmoles/kg/min adenosine (adenosine, Reanal) was infused (MTA, KUTESZ, Infumat, typ: 5157) into the vessel until a steady state was reached (approximately 2–3 min thereafter).

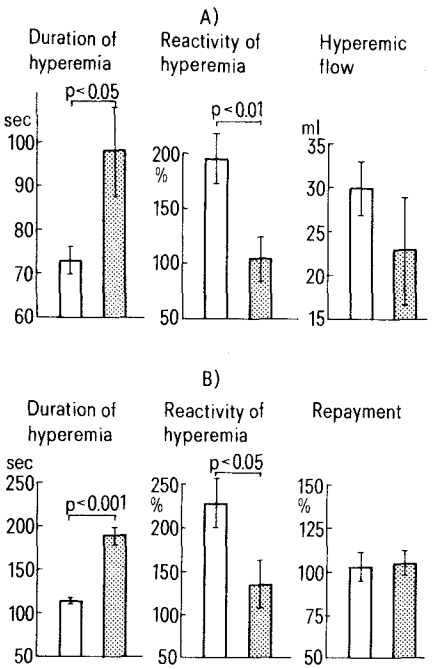

Figure 1. *A* Characteristics of reactive hyperemic response evoked by 1-min-asphyxia. *B* Characteristics of reactive hyperemic response evoked by 1-min-ligation of the coronary artery. □, Values (mean ± SEM) of metabolically healthy dogs (n = 14); ▨, values (mean ± SEM) of alloxan-diabetic animals (n = 12).

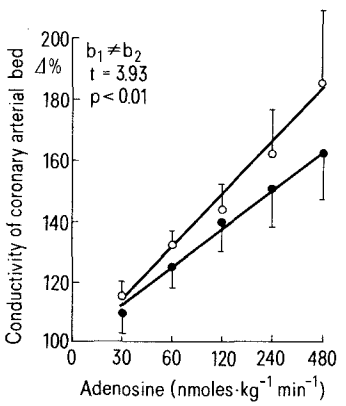

Figure 2. Conductivity of coronary arterial bed after adenosine infusion. ○, Values (mean ± SEM) of metabolically healthy dogs (n = 6); ●, values (mean ± SEM) of alloxan-diabetic animals (n = 6).

The conductivity of the coronary arterial bed was characterized by the ratio of flow to aortic pressure. Reactivity is characterized by the maximal blood flow related to the basal flow; the 'repayment of anoxia' is calculated by the ratio of the area of reactive hyperemic response and that of the flow debt caused by the coronary artery clamping[12]. The results (mean values ± SEM) were evaluated statistically using Student's paired and unpaired t-tests and also regression analysis.

Results and discussion. The plasma disappearance rate of glucose and body weight decreased after alloxan treatment, whereas the fasting plasma glucose level and urinary glucose excretion increased. No acetone was excreted, and blood urea nitrogen and coronary blood flow did not differ significantly from those of healthy animals. Mean arterial blood pressure was higher in alloxan-diabetic than in metabolically healthy dogs (table). The duration of the reactive hyperemic response caused either by asphyxia (fig. 1, A) or coronary clamping (fig. 1, B) increased significantly, while its reactivity to hypoxia decreased in alloxan-diabetic dogs, but there was no substantial difference in the degree of hyperemic flow between diabetic and healthy groups. During adenosine infusion the conductivity of the coronary arterial bed increased dose-dependently, but this was true to a lesser extent in alloxan-diabetic dogs than in healthy animals (fig. 2).

Recently, altered vascular reactivity has been demonstrated in cerebral vessels[4,13-14], in the skin[15] and in the femoral arterial bed[16] in diabetes. Our earlier[7] and present investigations showed an altered coronary response to catecholamine, to sympathetic stimulation and to hyperemic responses in diabetes. Macro- and microangiopathy of the vessels as a factor which might be responsible for these observations could be excluded histologically. The adrenergic vasomotor regulation has a considerable influence on the coronary arteries[17-19]; according to the metabolic state, the receptors can be of alpha or beta character[20]. Although this theory might also explain our results, it needs confirmation. Another possible explanation might be a change in ligand binding resulting from a change in the number of receptors, or the modification of receptor affinity often observed in endocrinological and metabolic diseases[21,22]. While a decrease in the number of beta adrenoceptors has been demonstrated[23] in diabetic rat heart, the decrease of the compliance in connective tissue[24,25] has also been held responsible for the modified diabetic vascular reactivity. The most probable suggestion[26] is that there is a change in the relation of receptors to the vessel wall in diabetes. The change in the reaction of the smooth muscle of coronary arteries shown in our results may be important in the modified diabetic vascular reactivity.

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Murine arylsulfatase C: Evidence for two isozymes[1]

K. Nelson, B.M. Keinanen and W.L. Daniel[2]

Department of Biochemistry, Albert Chandler School of Medicine, University of Kentucky, Lexington (Kentucky 40536, USA), and Department of Genetics and Development, University of Illinois, 515 Morrill Hall, 505 South Goodwin Avenue, Urbana (Illinois 61801, USA), October 6, 1982

Summary. SWR/J mice possess high arylsulfatase C, estrone sulfatase, and dehydroepiandrosterone sulfatase activities in liver, spleen, and kidney compared to A/J mice. This interstrain activity variation appears to be determined by at least 1 autosomal gene. Murine arylsulfatase C activity occurs in both hydrophobic and hydrophilic forms which differ with respect to certain biochemical properties and exhibit different subcellular distributions. The hydrophilic isozyme is a major component in kidney and brain extracts and a minor isozyme in liver and spleen extracts. The hydrophobic arylsulfatase C isozyme appears to be identical to steroid sulfatase. The hydrophilic arylsulfatase C isozyme does not possess steroid sulfatase activity; however, hydrophilic and hydrophobic arylsulfatase C share certain properties, suggesting that they may be structurally related. The autosomal gene(s) affects both arylsulfatase isozymes.

Arylsulfatase C (arylsulfate sulfohydrolase, EC 3.1.6.1) hydrolyzes sulfate ester bonds of a number of synthetic substrates, including p-nitrophenyl sulfate and 4-methylumbelliferyl sulfate, and appears to function as a steroid sulfatase in vivo. Arylsulfatase C (steroid sulfatase) deficiency is associated with X-linked ichthyosis[3], and the presumptive steroid sulfatase locus has been mapped to the distal short arm of the X-chromosome[4]. Unlike most known X-linked loci, both steroid sulfatase alleles are functional in female cells[5]. Relatively little is known regarding genetic regulation of normal arylsulfatase C activity in mammalian cells. We have developed a reliable sensitive assay for arylsulfatase C in crude preparations and have described interstrain and developmental variation of the murine enzyme[6]. This report presents data supporting the occurrence of 2 arylsulfatase C isozymes in murine tissues, describes their properties, and provides preliminary support for autosomal control of arylsulfatase C activity.

Methods. Inbred mice were purchased from the Jackson Laboratory, Bar Harbor, Maine and raised to 45 days of age. Tissues were homogenized in 9 vols of 0.2 M sodium phosphate buffer, pH 8.6, containing 1.0% (v/v) Triton X-100. The homogenates were sonicated and centrifuged at 20,000 × g for 30 min. The supernatant was used as the source of the enzyme unless otherwise indicated. Arylsulfatase C activity was determined using 4-methylumbelliferyl sulfate (MUS) as previously described[6]. Steroid sulfatase activity was estimated using estrone sulfate (E_1S) and dehydroepiandrosterone sulfate (DHEAS) as substrates. E_1S-sulfatase activity was measured using [6,7-^{3}H(N)]E_1S ammonium salt (New England Nuclear; 59 Ci/mmole) as substrate[7]. DHEAS-sulfatase activity was estimated using ^{3}H-DHEAS ammonium salt (NEN; 22.1 Ci/mmole) as substrate[8]. The ^{3}H-DHEA product was extracted directly into the scintillation cocktail. Zero time blanks were used for all assays. Subcellular localization of liver and kidney arylsulfatase C was explored using the method of Perumal and Robins[9] using 1 g of tissue. Arylsulfatase C was chromatographed on phenyl-Sepharose CL-4B (Pharmacia) using a method modified from that of Carson and Konigsberg[10]. 10% (w/v) homogenates (0.5 g tissue) were prepared in 0.05 M Tris-0.1 M NaCl-0.01% NaN_3 (Tris-saline-azide) buffer, pH 7.6, sonicated 15 sec, and centrifuged at 3000 × g for 20 min. The supernatant was applied to a 1 × 7 cm column of phenyl-Sepharose preequilibrated with Tris-saline-azide buffer, and the column was washed with 50 ml of the same buffer. The column was developed with 100 ml of Tris-saline-azide buffer containing 0.5% (v/v) Triton X-100. 1.5 ml fractions were collected and

Table 1. Arylsulfatase C activities from selected tissues of 45-day male and female mice

Strain	Sex	Liver	Kidney	Spleen	Brain
SWR/J	M	1.80±0.08	0.78±0.07	1.63±0.14	0.33±0.02
	F	1.77±0.16	0.71±0.06	1.73±0.10	0.30±0.01
C57BL/6J	M	2.06±0.17	0.37±0.04	1.00±0.11	0.22±0.02
	F	1.78±0.16	0.36±0.03	1.00±0.09	0.20±0.01
SJL/J	M	1.90±0.12	0.85±0.06	1.57±0.13	0.34±0.01
	F	1.70±0.18	0.76±0.10	1.37±0.16	0.32±0.03
A/J	M	1.03±0.10	0.33±0.03	0.78±0.06	0.25±0.03
	F	1.04±0.07	0.37±0.03	0.74±0.05	0.25±0.02

Activities are expressed as μmoles MU formed/g wet tissue/h. The mean and range of 5 animals are presented in each category.