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Involvement of Nitric Oxide in the Regulation of Regional Hemodynamics in Streptozotocin-Diabetic Rats

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Summary

In experimental and human diabetes mellitus, evidence for an impaired function of the vascular endothelium has been found and has been suggested to contribute to the development of vascular complications in this disease. The aim of the study was to evaluate possible regional hemodynamic *in vivo* differences between healthy and diabetic rats which would involve nitric oxide (NO). Central hemodynamics and regional blood flow (RBF) were studied using radioactive microspheres in early streptozotocin (STZ)-diabetic rats and compared to findings in healthy control animals. This method provides a possibility to study the total blood flow and vascular resistance (VR) in several different organs simultaneously. L-NAME iv induced widespread vasoconstriction to a similar extent in both groups. In the masseter muscle of both groups, acetylcholine 2 μg/kg per min, induced a RBF increase, which was abolished by pretreatment with L-NAME, suggesting NO as a mediator of vasodilation. In the heart muscle of both groups, acetylcholine alone was without effect while the combined infusion of acetylcholine and L-arginine induced an L-NAME-sensitive increase in RBF. The vasodilation induced by high-dose acetylcholine (10 μg/kg per min) in the kidney was more pronounced in the STZ-diabetic rats. The results indicate no reduction in basal vasodilating NO-tone in the circulation of early diabetic rats. The sensitivity to vasodilating effects of acetylcholine at the level of small resistance arterioles vary between tissues but was not impaired in the diabetic rats. In the heart muscle the availability of L-arginine was found to limit the vasodilatory effect of acetylcholine in both healthy and diabetic rats. In conclusion, the results indicate a normal action of NO in the investigated tissues of the early STZ-diabetic rat.

Key words
Blood flow • Diabetes mellitus • Hemodynamics • Nitric oxide • Rat

Introduction

The vascular endothelial cells have the capacity to release several vasoactive substances such as prostaglandins, NO, endothelium-derived hyperpolarizing factor and the peptide endothelin. Continuous formation of NO contributes to maintaining patency of the blood vessels and several stimuli such as acetylcholine, bradykinin and shear stress induce vasodilation, which is dependent on the intact function of vascular endothelium (Lüscher and Noll 1995).

In diabetes mellitus, an impaired endothelium-dependent vasodilation has been found in humans with type 1 as well as type 2 diabetes, mainly demonstrated using venous occlusion plethysmography and stimulation with acetylcholine or methacholine (Makimattila *et al.*

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1996, O´Driscoll *et al.* 1997, Watts *et al.* 1996, Williams *et al.* 1996). However, other studies in humans using the same technique yielded conflicting results (Calver *et al.* 1992, Smits *et al.* 1993, Gazis *et al.* 1999). In STZ-induced experimental diabetes, alterations of vascular endothelial function have been demonstrated in isolated vessel segments of aorta and mesenteric resistance arteries (Kamata *et al.* 1989, Diederich *et al.* 1994) as well as in skeletal muscle and pial arterioles (Hill and Ege 1994, Mayhan *et al.* 1991). Also in other models of experimental diabetes, changes in vascular endothelial function have been found (Matsunaga *et al.* 1996, Tesfamariam *et al.* 1989). However, opposing findings have been presented both *in vitro* (Mulhern and Docherty 1989) and *in vivo* (Kiff *et al.* 1991 a, Brands and Fitzgerald 1998).

In diabetes, evidence for a reduced NO bioactivity has been found (De Vriese *et al.* 2000) either due to a decreased production of basal NO, a reduced availability of the substrate L-arginine for the NO-producing enzyme, the nitric oxide synthase (NOS) (Pieper and Peltier 1995) or an increased destruction of NO by oxygen free radicals (Diederich *et al.* 1994, Rubanyi and Vanhoutte 1986). It has been suggested that an altered action of the vascular endothelial cells might contribute to the development of diabetic vascular complications (Pieper 1998). However, the results of previous studies in diabetic patients and experimental models of diabetes have not been consistent. Most studies have focused on conduit and large resistance arteries although diabetic vascular changes also affect smaller blood vessels (the diabetic microangiopathy). With the microsphere method for direct *in vivo* measurement of RBF, changes in nutritive blood flow which reflect alterations at the level of small resistance arterioles, can be measured. Previously, the microsphere method has been used for evaluating NO involvement in blood flow regulation of the hypertensive rat (Granstam *et al.* 1998). In the present study, RBF in the kidney, heart, skeletal muscle and brain has been measured in the STZ-diabetic rat during pharmacological intervention with the NO-system using NOS inhibition and stimulation with acetylcholine and L-arginine and compared to findings in non-diabetic control rats.

Methods

Surgical procedures

All experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe no 123, Strasbourg 1985). The studies were approved by the Animal Ethics Committe of the University of Uppsala. A total of 56 Sprague-Dawley rats obtained from Möllegaard (Denmark) were included in this study. Twenty-seven animals were made diabetic with an iv injection of streptozotocin (40 mg/kg STZ, Zanosar®, Pharmacia Upjohn, MI, USA) and 29 were healthy control rats. Additional 20 rats were excluded due to technical difficulties during surgery or failure of STZ administration. The rats in the diabetic groups were fasted overnight before STZ administration and buprenorphin (Temgesic®, Meda Sverige AB, Sweden) was used as an analgesic during the injection. One week after the STZ injection, blood glucose levels were determined by tail vein samples (Hemocue®, Sweden) and all rats with blood glucose levels greater than 20 mmol/l were considered diabetic and were included in the study. Blood glucose was 32±1 mmol/l one week after STZ administration and 33±1 mmol/l during the experiment. In the healthy group blood glucose was 6.0±0.1 mmol/l. Blood flow determinations were performed 29±2 days after STZ administration, i.e. after 3 weeks of established hyperglycemia. The age of the STZ-treated rats was 13±0.5 weeks at the time of experiment. The control rats were age-matched (12±0.4 weeks) (NS between groups). Animal weights were 0.35±0.01 kg for the STZ-diabetic rats and 0.42±0.01 kg for the control rats ($P<0.001$ between groups).

On the day of blood flow determination, the animals were anaesthetized with thiobutabarbital, 120 mg/kg ip (Inactin®, RBI Co, USA). The animals were tracheostomized and breathed spontaneously. Both femoral arteries were cannulated with polyethylene tubing for continuous blood pressure and heart rate recordings and for the collection of reference blood samples. One femoral vein was cannulated for the infusion of drugs. A polyethylene tube was placed into the left heart ventricle *via* the right common carotid artery. At the end of surgical procedure, heparin (500 IU/kg bw, Lövens, Denmark) was given to prevent clotting in the tubes. During the surgical preparation, a slow infusion of saline (5 ml/kg per hour for the normal rats and 10 ml/kg per hour for the diabetic animals) was administered to avoid dehydration and maintain normal hematocrit levels (46.6±0.3% and 46.2±0.7 % in the normal and diabetic rats, respectively, immediately before the microsphere procedure). There was no urinary secretion of acetoacetate (Redia-test®, Boehringer

Ingelheim). Acid-base data (ABL 300 and Osm 3; Radiometer, Copenhagen, Denmark) for the diabetic and healthy rats before microsphere injections were not significantly different: pH 7.38±0.01 vs. 7.40±0.01, PCO_2 5.8±0.1 vs. 5.6±0.1 kPa, PO_2 10.4±0.4 vs. 11.6±0.4 kPa, base-excess was 0.7± 0.6 vs. 1.1± 0.5. Neither the diabetic nor the healthy rats were hypoxic or acidotic at the time of the experiment, indicating a satisfactory general condition during anesthesia.

Blood flow determination

Regional blood flow was determined with 15 µm radioactive microspheres (DuMedical Scandinavia AB, Sweden) (Granstam *et al.* 1998). Spheres labelled with three different radionuclides, ^{141}Ce, ^{103}Ru and ^{95}Nb were used, allowing three blood flow determinations in each animal. Approximately 150 000 spheres dispersed in a total volume of 0.3 ml saline (containing 0.01 % Tween without dextrane) were administered at each injection. A small volume of blood (less than 0.1 ml) was aspirated from the heart immediately before the sphere injection to ensure a homogenous solution. The spheres were injected over 15 s and the sampling of the reference blood was started at the same time and continued for 1 min. The reference blood sample was obtained by continuous aspiration from the artery tubing *via* a peristaltic pump (P-1, Pharmacia, Sweden) at a rate of approximately 0.6 ml/min. The volume lost during reference sampling was compensated for by the sphere volume injected in addition to the continuous infusion of saline. After each sphere injection, 0.1 ml blood was again aspirated from the heart catheter and diluted to about 0.5 ml, to measure the spheres trapped in the tubing. The exact amount of radioactivity given could be calculated by taking aliquots (10 µl) of the initial sphere volume and of the diluted aspirated volume after sphere injection for gamma counting.

At the end of the experiments, the animals were given an overdose of anesthesia iv followed by a KCl injection (Sigma-Aldrich, Stockholm, Sweden).Tissue samples were taken from the kidneys (whole), the masseter muscle, apical left ventricle of the heart, left brain hemisphere and left brainstem. The blood and tissue samples were weighted and then all samples were analyzed in a three-channel gamma-spectrometer (modified model from Nuclear Chicago, USA). Background activity and cross-over between energy channels were considered for each sphere injection.

RBF was calculated by multiplying tissue CPM (counts per minute) with the reference flow (as g blood per min) and dividing with the CPM of the reference blood sample. RBF was related to tissue weight and expressed as g/min per g tissue(tw). Cardiac output (CO) was calculated by multiplying the total amount of radioactivity administered with the reference flow, divided by the radioactivity of the reference blood sample. Cardiac index (CI) was defined as CO/body weight (bw) expressed as g/min·kg(bw). Total peripheral vascular resistance (TPRI) was calculated as TPRI = MAP/CI; MAP = mean arterial blood pressure during sphere injection. TPRI was expressed as mmHg·min·kg(bw)/g. Regional vascular resistance (VR) = MAP/RBF, was expressed as mm Hg·min·g(tw)/g.

Experimental protocols

In the initial series of experiments, the first injection of microspheres for the measurement of resting RBF was performed after a 15-min stabilization period. Five minutes later, a bolus injection of L-NAME (10 mg/kg) was administered which was followed by an iv infusion of L-NAME (10 mg/kg per hour) at a rate of 2 ml/h. RBF was then determined after 10 and 30 min of L-NAME infusion, respectively (control rats n=8; diabetic rats n=7).

In the second series of experiments, resting RBF was also determined. Five min later, an iv infusion of acetylcholine, 2 µg/kg per min (ACH 2) was started and maintained throughout the experiment using an infusion pump (P-2000, IVAC Medical Systems, UK) at a rate of 0.1 ml/min. After 6 min of acetylcholine infusion, the second RBF determination was performed. After an additional 5 min, iv infusion of L-arginine, 150 mg/kg per min, was added using another infusion pump at a rate of 0.2 ml/min (ACH 2+L-arg). Following 3 min of the combined infusion of acetylcholine and L-arginine, the third blood flow determination was performed (control rats n=8; diabetic rats n=8).

In the third series of experiments, after measurement of baseline RBF, an iv infusion of a higher dose of acetylcholine, 10 µg/kg per min (ACH 10), was initiated. RBF was determined after 5 min of infusion (control rats n=6; diabetic rats n=7). In 4 rats in each group, iv infusion of L-arginine, 150 mg/kg per min, was added using the second infusion pump and a third blood flow determination was performed after 3 min of combined infusion of acetylcholine and L-arginine (ACH 10+L-arg). In 2 control rats and 3 diabetic rats L-NAME was administered using the sequence of bolus injection followed by iv infusion described above and the final

RBF registration was made after 10 min of combined high-dose acetylcholine and L-NAME.

In the fourth series of experiments, animals were pretreated with L-NAME using the sequence of bolus injection followed by iv infusion described above. After 10 min of L-NAME infusion, the first microsphere injection was performed. Five minutes later, iv infusion of acetylcholine, 2 µg/kg per min, was started and maintained until being replaced by L-arginine, 150 mg/kg per min. After 6 min of combined infusion of L-NAME and acetylcholine, RBF was determined with microspheres. Finally, acetylcholine was exchanged for L-arginine and after 3 min of combined infusion of L-NAME and L-arginine, radioactive microspheres were administered (control rats n=7; diabetic rats n=5).

Drugs

Acetylcholine (acetylcholine chloride), L-NAME (N^{ω}-nitro-L-arginine methyl ester), L-arginine (L-arginine hydrochloride) from Sigma-Aldrich, Sweden were dissolved in saline.

Statistical analysis

Statistical analysis was performed with non-parametric tests. Between animal groups Mann Whitney U test was applied, while Wilcoxon signed rank test was used within each group. The non-parametric tests were chosen because the group sizes were not large enough to properly evaluate the presence of a normal distribution. Data from measurements during resting conditions were pooled from series 1, 2 and 3. All values are given as the mean ± S.E.M. P<0.05 was regarded as significant. NS indicates not significantly different.

Results

Resting conditions

Under resting conditions MAP was 114±4 mmHg in control rats (n=22) and 120±3 mmHg in the STZ-diabetic group (n=22, NS between groups). TPRI was 0.36±0.02 and 0.42±0.02 mmHg·min·kg(bw)/g, respectively, whereas CI was 328±16 and 303±15 g/min·kg(bw), respectively (NS between groups). Heart rate was significantly lower among the diabetic animals: 302±15 compared to 345±8 beats/min (P<0.001). RBF in the kidneys and masseter muscle was lower and regional vascular resistance (VR) was significantly higher in the hyperglycemic rats compared to the control animals (Fig. 1). In the heart, RBF and VR were similar in both groups (Fig. 1). In the brain hemisphere and brainstem, RBF was not significantly different between the control and diabetic rats but VR was slightly higher in the diabetic group: 140±10 vs 179±14 in the hemisphere and 118±11 vs 155±15 mm Hg·min·g tissue/g (P<0.05 between groups).

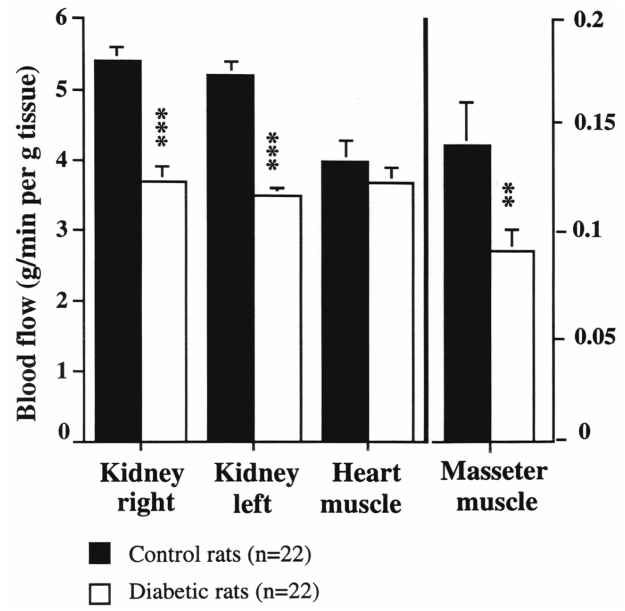

Fig. 1. *RBF in the kidneys, heart and masseter muscles under resting conditions. Data were summarized from series 1 (baseline measurement before iv L-NAME), series 2 (baseline before iv acetylcholine 2 µg/kg per min) and series 3 (baseline before iv acetylcholine 10 µg/kg per min). Full column – control rats (n=22), open column – diabetic rats (n=22). **P<0.01, ***P<0.001.*

L-NAME

Intravenous administration of L-NAME as a bolus injection followed by a slow iv infusion, induced significant increases of MAP and TPRI in both groups. After 10 min of L-NAME infusion, MAP was increased by 40±9 % (P<0.05) in the normal rats and 25±4 % (P<0.05) in the diabetic rats compared to control. The corresponding values for the TPRI were +205±22 % (P<0.05) and +126±23 % (P<0.05). At 30 min, the increase in MAP as well as the increase in TPRI were significantly larger in the normal rats (+43±7 % and +359±33 %, respectively) compared to the hyperglycemic animals (+16±5 % and +152±24 %; P<0.05 between groups). A corresponding reduction in CI by 41-68 % was observed in both groups, whereas heart rate was

unaffected by L-NAME. In the kidney and masseter muscle administration of L-NAME induced a reduction in RBF and an increase of VR (Table 1). The effect of L-NAME was similar between the control and STZ-diabetic animals. In the heart muscle, only minor RBF reduction was observed. In the brain hemisphere and brainstem iv administration of L-NAME significantly reduced RBF (P<0.05, Table 1) and induced a significant increase (P<0.05) in VR, indicating widespread vasoconstriction. In the normoglycemic animals, the effect of L-NAME was more pronounced after 30 min infusion compared to 10-min data, whereas in the STZ-diabetic rats, maximal vasoconstriction was already achieved after 10 min of L-NAME infusion.

Table 1. Effect of L-NAME, a bolus injection of 10 mg/kg followed by iv infusion of 10 mg/kg per hour on regional blood flow (g/min per g tissue) in control rats (n=8) and streptozotocin-diabetic rats (diabetic, n=7).

Tissue	**Rat group**	**Resting conditions**	**10 min L-NAME**	**30 min L-NAME**
Kidney (right)	*control*	4.85±0.33	1.94±0.06*	2.05±0.20*
	diabetic	4.00±0.30	1.83±0.13*	1.45±0.16*,†
Masseter muscle	*control*	0.17±0.04	0.09±0.01*	0.06±0.01*
	diabetic	0.13±0.02	0.09±0.01	0.07±0.01*
Heart muscle	*control*	3.85±0.43	4.02±0.36	3.60±0.34†
	diabetic	3.54±0.36	3.58±0.23	3.15±0.23
Left hemisphere	*control*	1.04±0.14	0.64±0.03*	0.48±0.01*,†
	diabetic	0.77±0.07	0.49±0.01*	0.43±0.02*
Left brainstem	*control*	1.30±0.24	0.59±0.05*	0.41±0.05*,†
	diabetic	0.89±0.15	0.44±0.04*	0.38±0.04*

*All values are given as mean ± S.E.M. * P<0.05 Wilcoxon signed rank test within groups compared to resting conditions, † P<0.05 Wilcoxon signed rank test, blood flow after 30 min iv L-NAME compared to blood flow after 10 min iv L-NAME.*

Acetylcholine and L-arginine

Central hemodynamics

Infusion of acetylcholine, 2 µg/kg per min, induced a significant reduction in MAP in the normoglycemic rats, –13±5 % (P<0.05; n=8), whereas in the STZ-diabetic animals, MAP was unaffected by acetylcholine, –4±5 % (n=8). In the either group of rats, neither TPRI, CI nor heart rate were significantly affected by acetylcholine 2 µg/kg per min.

During the combined infusion of acetylcholine 2 µg/kg per min and L-arginine, 150 mg/kg per min, MAP was reduced compared to resting conditions by 13±6 % (P<0.05) in the control rats and by 20±8% (NS) in the diabetic rats. In the diabetic rats, TPRI was reduced by 34±7 % (P<0.05) and CI was significantly increased by 23±6 % (P<0.05). In the control rats, there were no significant changes in either TPRI or CI.

Infusion of acetylcholine, 10 µg/kg per min, reduced MAP 27±2 % (P<0.05) in the control rats and by 34±4 % (P<0.05) in the hyperglycemic rats. TPRI was reduced by 24±6 % (P<0.05) and 40±7 % (P<0.05), respectively. CI and heart rate were not significantly affected. In those animals, in which L-arginine was added to the infusion of high-dose acetylcholine, the reduction in MAP was maintained, whereas in those animals, which received L-NAME, MAP returned to baseline levels.

Infusion of acetylcholine in animals pretreated with L-NAME slightly reduced MAP in both groups, –6±1 % (P<0.05; n=7) and –7±4 % (n=5) in control and diabetic rats, respectively. TPRI, CI and heart rate were not significantly affected in either group.

Kidney

In the kidney of normoglycemic rats, iv infusion of acetylcholine (2 µg/kg per min) and the combined infusion of acetylcholine and L-arginine did not

significantly affect RBF or VR. In the diabetic rats, on the other hand, a slight increase in RBF and a significant reduction in VR was observed during the combined administration of acetylcholine 2 µg/kg per min and L-arginine (Fig. 2a). Intravenous administration of acetylcholine (10 µg/kg per min) significantly increased RBF and reduced VR, indicating vasodilation (Fig. 2b). The effect was significantly more pronounced in the STZ-diabetic animals. No further effect was achieved by the addition of L-arginine to high-dose aceylcholine. In animals pretreated with L-NAME, there were no changes in either RBF or VR in control or hyperglycemic rats.

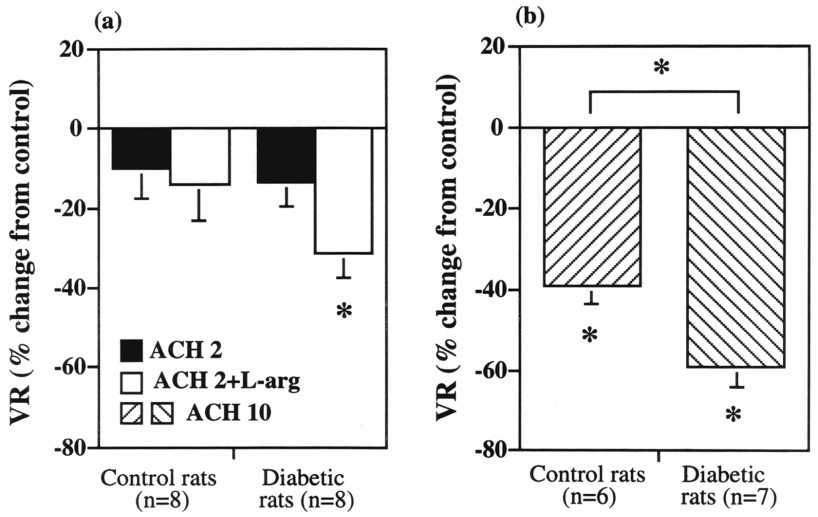

Fig. 2. **A.** *Change in VR (% from control) in the kidney during iv infusion of acetylcholine 2 µg/kg per min (ACH 2, full column) and the combined infusion of acetylcholine 2 µg/kg per min and L-arginine 150 mg/kg per min (ACH 2+L-arg, open column). Control rats (n=8), diabetic rats (n=8).* **B.** *In a separate series of experiments, change in vascular resistance (% from control) induced by acetylcholine 10 µg/kg per min was investigated: Right hatched column – control rats (n=6), left hatched column – diabetic rats (n=7). *P<0.05*

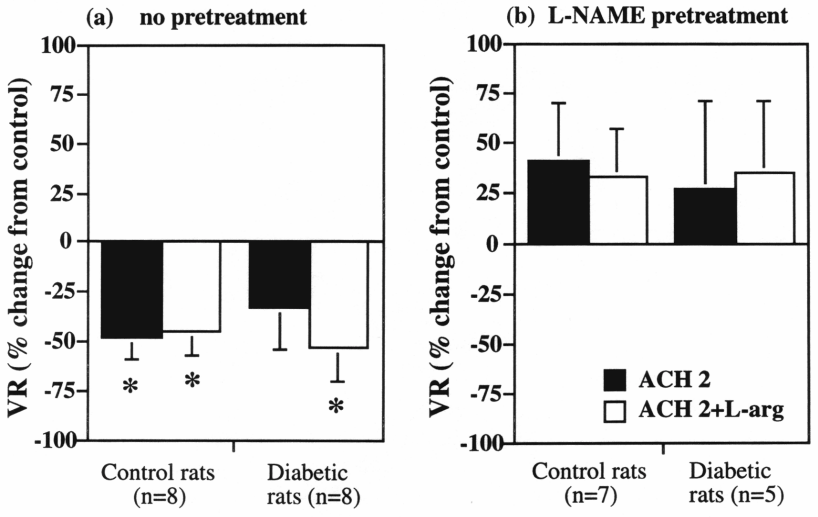

Fig. 3. *Change in VR (% from control) in the masseter muscle during infusion of acetylcholine 2 µg/kg per min (ACH 2, full column) and the combined infusion of acetylcholine 2 µg/kg per min and L-arginine 150 mg/kg per min (ACH 2+L-arg, open column) in animals without pretreatment (control rats n=8, diabetic rats n=8, Fig. 3a) and in animals pretreated with L-NAME (control rats n=7, diabetic rats n=5, Fig 3b). *P<0.05.*

Masseter muscle

Intravenous infusion of low-dose acetylcholine and the combined infusion of low-dose acetylcholine and L-arginine significantly increased RBF in the masseter muscle in both control and diabetic rats (P<0.05). VR during low-dose acetylcholine and L-arginine was reduced in both groups (P<0.05, Fig. 3a), indicating vasodilation. In animals pretreated with the NOS inhibitor L-NAME, no significant changes in RBF or VR were observed in either rat group (Fig. 3b), suggesting NO as a mediator of the acetylcholine-induced vasodilation. In both groups, there was a tendency towards the increase in VR following acetylcholine infusion during NOS inhibition.

Heart muscle

In the apex of the left heart ventricle, iv administration of acetylcholine alone, 2 µg/kg per min, did not significantly affect RBF. VR was slightly reduced (Fig. 4a). However, during the combined infusion of acetylcholine and L-arginine, a significant increase in RBF and a reduction in VR were observed in both groups of rats (Fig. 4a). In the animals pretreated with L-NAME, no significant changes in regional hemodynamics were observed in either control or diabetic rats following either acetylcholine 2 µg/kg per min or the combined infusion of low-dose acetylcholine and L-arginine (Fig. 4b). No additional effect was achieved with acetylcholine 10 µg/kg per min (data not shown).

Brain

RBF measurements in the left brain hemisphere and brainstem did not reveal any significant changes in cerebral perfusion during infusion of low-dose acetylcholine or the combined infusion of acetylcholine 2 µg/kg per min and L-arginine (data not shown). During infusion of high dose of acetylcholine (10 µg/kg per min) a slight but significant reduction in left hemisphere RBF was observed in the control group (0.74±0.08 to 0.61±0.03 g/min per g tw). In the STZ-rats the RBF was not significantly changed (0.55±0.06 to 0.51±0.04 g/min per g tw).

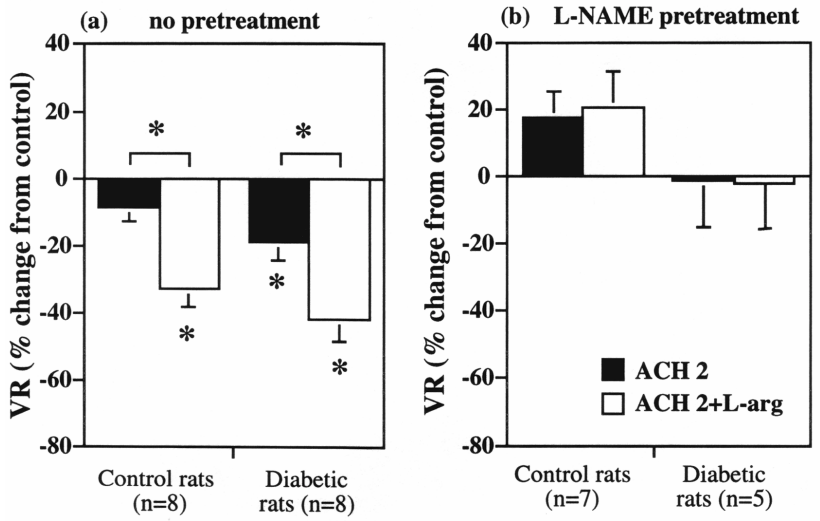

Fig. 4. *Change in VR (% from control) in the heart muscle during infusion of acetylcholine 2 µg/kg per min (ACH 2, full column) and the combined infusion of acetylcholine 2 µg/kg per min and L-arginine 150 mg/kg per min (ACH 2+L-arg, open column) in animals without pretreatment (control rats n=8, diabetic rats n=8, Fig. 4a) and in animals pretreated with L-NAME (control rats n=7, diabetic rats n=5, Fig. 4b). *P<0.05.*

Discussion

In the present study, analysis of central hemodynamics under resting, normovolumic conditions, showed similar MAP, TPRI and CI between normoglycemic and STZ-diabetic rats, whereas heart rate was significantly reduced in the diabetic animals. A decrease in cardiac output has already been observed within the first week of STZ-induced diabetes mellitus (Brands *et al.* 2000). In a study of diabetic animals 4 weeks after STZ administration, MAP and TPRI were reduced, whereas CI was increased (Litwin *et al.* 1991). After 12 weeks of disease duration, normotension was reestablished by an increase of cardiac output in response to the reduced TPRI (Carbonell *et al.* 1987). Heart rate was simultaneously reduced while stroke volume was increased (Carbonell *et al.* 1987). Normal MAP and bradycardia was also observed in STZ-diabetic Wistar rats (Kiff *et al.* 1991 a) which is well in line with the findings of the present study. Thus, the duration of diabetes is clearly of major importance for alteration of systemic hemodynamics.

Evaluation of resting regional hemodynamics revealed reduced RBF in the kidneys and skeletal muscle of the early STZ-diabetic rat compared to the normoglycemic control rat. This is in accordance with previous studies in the diabetic rat, in which a reduction in hindquarter and renal RBF has been observed (Brands *et al.* 2000, Brands and Hopkins 1996), although regional vasoconstriction might be differently distributed (Kiff *et al.* 1991a). The reason for the reduction in resting renal and skeletal muscle RBF is not clear. Increased production of endothelium-derived vasoconstrictors, such as prostanoids, has been found both under resting conditions (Tesfamariam *et al.* 1989) and following stimulation with acetylcholine (Mayhan *et al.* 1991, Ito *et al.* 1991, Noll *et al.* 1997) and has been suggested to oppose the effect of endothelium-derived relaxing factors

on the vascular smooth muscle cells. Subnormal circulating levels of insulin in the STZ-diabetic rat might also contribute to the reduction in RBF. A vasodilating effect of physiological plasma insulin concentrations has been found (Utriainen *et al.* 1995). STZ-induced diabetes is the result of reduced insulin production, which is, however, not completely abolished since ketoacidosis did not develop in our experimental set-up.

Continuous formation and release of NO from the vascular endothelial cells contribute to maintain blood vessels patent. In the present study, administration of the NOS inhibitor L-NAME was found to induce widespread vasoconstriction in both groups of animals, indicating the presence of a basal vasodilating NO-tone of similar magnitude in the investigated tissues of diabetic and control rats. This finding is in accordance with previous results from the rat (Kiff *et al.* 1991a, Fouyas *et al.* 1996) and humans (Catalano *et al.* 1997), although conflicting results in humans also exist (Calver *et al.* 1992). It has been suggested that an unaffected endothelial NO synthesis might be of importance to prevent hypertension at the onset of diabetes (Fitzgerald and Brands 2000, Claxton *et al.* 2000).

Administration of acetylcholine has three primary effects on the cardiovascular system: vasodilation, negative chronotropic and negative inotropic effects (Taylor 1985). Vasodilatation occurs through the activation of muscarinic receptors located on the vascular endothelial cells (Furchgott and Zawadzki 1980). In the present *in vivo* study, the doses of iv administered acetylcholine were carefully chosen to produce limited reductions in MAP to avoid baroreceptor reflex sympathetic activation and to allow the evaluation of the local vasodilatory response to acetylcholine. The limited alteration in heart rate suggests no major general baroreflex activation, but does not completely rule out some regional sympathetic nerve stimulation, which could reduce the magnitude of vasodilation seen following acetylcholine administration.

In the masseter muscle, both administration of acetylcholine and the combination of acetylcholine and L-arginine induced a vasodilation, which was abolished by pretreatment with L-NAME, suggesting NO as a mediator of the vasodilation. The effect was not significantly different between groups. In the kidney, high-dose acetylcholine induced a vasodilation which was slightly more pronounced in the diabetic animals. These results indicate a normal action of NO in these tissues in the early STZ-diabetic rat. This finding is in contrast with other studies in STZ-diabetic rats, in which an impaired endothelium-dependent vasodilation has been found (Kamata *et al.* 1989, Diederich *et al.* 1994, Hill and Ege 1994, Mayhan *et al.* 1991). However, several *in vivo* studies in this model (Kiff *et al.* 1991a, Brands and Fitzgerald 1998) have demonstrated a normal vascular endothelial cell function, which was unaffected by chronic hyperglycemia. In the studies by Kiff *et al.* (1991ab) and Brands and Fitzgerald (1998), the *in vivo* measurements were performed with chronically implanted pulsed Doppler probes. Interestingly, regional heterogeneity of endothelium-dependent vasodilation was demonstrated with this technique; bradykinin- but not acetylcholine-mediated vasodilation was depressed in the hindquarters vasculature of diabetic rats but was normal in the kidney and mesenterium (Kiff *et al.* 1991b). With the microsphere technique, applied in the present study, nutritive blood flow to different tissues *in vivo* is measured. Changes in nutritive blood flow possibly reflect alterations at the level of small resistance arterioles, which are difficult to study using an *in vitro* or other *in vivo* approaches. Another possible explanation for the differing results might be the duration of diabetes. Other studies in early STZ-diabetic animals have revealed a normal vascular endothelial function (Brands and Fitzgerald 1998, Claxton *et al.* 2000). Diederich *et al.* (1994) found that endothelium-dependent vasodilation in mesenteric arteries was increasingly attenuated with the duration of disease. In isolated aortic rings from STZ-diabetic rats, endothelium-dependent vasodilation was enhanced or unaltered in vessel segments from animals with diabetes of short duration (up to 2 weeks) whereas endothelium-dependent vasodilation was impaired if duration of disease was longer (8 weeks) (Pieper 1999). The present experiments were performed after 3 weeks of established hyperglycemia. This limited duration of disease might not induce irreversible diabetic changes in the vascular endothelium.

In the heart muscle, the present study demonstrated that acetylcholine alone did not significantly affect RBF or VR. Interestingly, the combined infusion of acetylcholine and L-arginine induced regional vasodilation. The result was similar in diabetic and control animals. L-arginine is the amino acid substrate for NOS. Plasma L-arginine concentrations have been found to be reduced in STZ-diabetic rats (Pieper and Peltier 1995) and some diabetic patients (Hagenfeldt *et al.* 1989, Grill *et al.* 1992). *In vitro* studies of isolated aortic rings from STZ-diabetic rats have demonstrated an improved endothelial function following pretreatment with L-arginine (Pieper *et al.* 1996). The

findings of the present study suggest that regional vascular differences exist concerning the need for additional substrate for NO synthesis both in control and STZ-diabetic rats. In diabetic dogs, intracoronary infusion of L-arginine improved endothelium-dependent relaxation in spite of a normal plasma arginine concentration (Matsunaga *et al.* 1996), suggesting that static plasma L-arginine levels need not to be reduced for supplemental L-arginine to affect endothelial function. This further indicates that additional substrate for NO formation could be of importance for the magnitude of NO-induced vasodilation in the coronary circulation.

Acetylcholine was found to induce only minor changes of RBF in the brain in either group of animals. In a previous microsphere study in the spontaneously hypertensive rat, cerebral RBF was unaffected by iv administration of a similar dose of acetylcholine (Granstam *et al.* 1998), suggesting preserved cerebral RBF autoregulation under the experimental conditions of these studies. During infusion of the highest dose of acetylcholine, slight RBF reductions were observed in both groups. This dose of acetylcholine reduced MAP more markedly, probably to the lower limit of the cerebral autoregulatory curve, explaining some of the reduction in RBF.

In conclusion, the results of the present investigation indicate a similar, or in the renal circulation somewhat enhanced, basal vasodilating NO-tone in the circulation of STZ-diabetic compared to healthy rats. The sensitivity to vasodilating acetylcholine varied between tissues, but no generally impaired NO activity was found in diabetic rats. In some tissues such as the heart muscle, the availability of L-arginine was found to limit the vasodilatory effect of acetylcholine, whereas the evidence for a slightly more pronounced effect of acetylcholine in the STZ-diabetic rat was found in the kidney. However, our results evaluated a hyperglycemic condition with a duration of three weeks. The duration can be of major importance when interpreting the findings.

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