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Recovery of microvascular responses during streptozotocin-induced diabetes

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Abstract

Microvascular reactivity of cannulated and pressurised rat cremaster arterioles was studied during the progress of diabetes using mechanical (intraluminal pressure) and chemical (acetylcholine, sodium nitroprusside) stimulation. Microvessels were studied in controls and at 2, 4 and 8 weeks following induction of diabetes by streptozotocin. Mechanical responses were stable at the test pressure (70 mmHg) used for pharmacological investigations during the period of diabetes. Acetylcholine application could induce maximal dilatation in control vessels and in vessels exposed to 8 weeks of diabetes. However, acetylcholine administration failed to generate maximal dilatation at 2 and 4 weeks of diabetes. During the period of diabetes, loss of nitric oxide (NO) pathway effectiveness was revealed by diminished response to sodium nitroprusside and by reduced capacity of *N*ω-nitro-L-arginine methyl ester (L-NAME) to decrease resting diameter and acetylcholine-evoked dilatation. L-NAME and indomethacin application revealed a significant non-NO, non-prostaglandin contribution to the acetylcholine response at 4 and 8 weeks of diabetes. Recovery of responsiveness to acetylcholine and stabilisation of resting vessel diameter during diabetes may, in part, be due to increasing effectiveness of non-NO, non-prostaglandin pathways.

Keywords: Streptozotocin-induced diabetes; Arteriole; Endothelium; Smooth muscle; Nitric oxide (NO); Prostaglandin

1. Introduction

Microvascular dysfunction remains a significant contributor to the increased morbidity and mortality associated with long-term diabetes mellitus. While the Diabetes Control and Complications Trial (1994) has implicated a primary role for hyperglycemia in the etiology of microangiopathy, it is evident that until methods are available for maintaining near perfect metabolic control, a certain proportion of the diabetic population will be at risk of developing microvascular diseases. In relation to the natural course of microangiopathy, it appears that structural alterations of the microvasculature are preceded by functional microcirculatory disturbances, which result in inappropriate levels of perfusion of the tissue implying that the normal, local haemodynamic regulatory mechanisms are impaired or overridden (Larkins and Dunlop, 1992; Tooke, 1995).

Studies performed on diabetic patients or animals have shown compromised relaxation in the vasculature. However, there is variation between different observations regarding the timecourse of these pathophysiological responses and the vessel wall level (e.g. endothelium, smooth muscle) affected. For example, in human type I diabetes, O'Driscoll et al., 1997 demonstrated dysfunction of endothelial-dependent vasodilatation. On the other hand, others reported an irregularity in the endothelium-independent relaxation (Khan et al., 1996). Regarding type II diabetes, the available data is also contradictory. Some groups have implicated insufficient vasodilatation depending on endothelial dysfunction (Hogikyan et al., 1998), while others have revealed an impairment both in endothelial-dependent and -independent relaxation (Watts et al., 1996; Williams et al., 1996).

In animals, particularly in streptozotocin-treated rats, there is experimental evidence for an impaired endothelium-dependent relaxation, while the endothelium-independent vasodilatation remains unaltered (Taylor et al., 1995; Kamata and Kondoh, 1996; Pieper, 1999). On the other

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hand, some suggest that short-term (Brands and Fitzgerald, 1998) as well as long-term (Kappagoda et al., 1989) diabetes has not altered (Sexl et al., 1995) or, in some cases, even enhanced (Heygate et al., 1996), the endothelium-dependent relaxation of the blood vessels. Generally, in larger vessels, responses to acetylcholine have been described as unchanged, reduced or enhanced depending on the particular point in time chosen following the administration of streptozotocin (Chang and Stevens, 1992; Taylor et al., 1994; Hopfner et al., 1999; Kobayashi and Kamata, 1999; Pieper, 1999). Similar time-dependent changes have also been observed in microvessels (Furman and Sneddon, 1993; Ralevic et al., 1995; Heygate et al., 1996; Crijns et al., 1998).

It is evident that an impaired vasodilatation in diabetes leads to failing tissue perfusion and clinically relevant complications (e.g. retinopathy, nephropathy and possibly neuropathy). Understanding the nature and time course of mechanisms underlying altered microvascular dysfunction in animals may provide information to identify sites for pharmacological interventions and contribute to studies aiming to prevent the development of human microvascular abnormalities. Using an isolated arteriole preparation, the present studies were designed to contribute to our knowledge about the time course of pathophysiological changes in the microcirculation. We have studied endothelial-dependent and endothelial-independent mechanisms in microvessels during an 8-week period following the induction of diabetes in rats.

We hypothesize the following that, first, an impairment in vasodilatory pathways, e.g. due to either reduced effectiveness of nitric oxide (NO) or/and relaxing prostanoids does exist; second, an increase of imbalance between constrictor and dilator pathways is apparent; and third, the importance of alternative pathways is augmented by the development of diabetes and can contribute to recovery of dilatory responses.

2. Materials and methods

2.1. Operative procedures

Experiments were performed on age-matched male Sprague–Dawley rats (6, 8, 10 and 14 weeks). The animals were kept under standardized environmental conditions (22°C, 60% humidity, artificial light from 0600 to 1800 h) in the animal quarters for at least 5 days prior to the experiments. During this period, the rats were allowed free access to standard rat chow and drinking water. The study was approved by the Animal Ethics Committee at Royal Melbourne Institute of Technology University.

In all animals, anesthesia was induced with a single dose of thiopental sodium i.p. (100 mg/kg body wt.). The anesthetized animals were placed on an operating table, the cremaster muscle was excised and immediately placed in a cold (4°C) bath (Falcone et al., 1993). An arterial blood sample was collected for $[Glu]_B$ analyses from control animals and 2, 4 and 8 week diabetic animals (control 5.5 ± 0.2, 2 weeks 22.3 ± 0.6, 4 weeks 22.7 ± 0.5 and 8 weeks 22.9 ± 0.5 mmol/l). A blood glucose level satisfying the requirements published by the American Diabetes Association 1998 (Dinneen et al., 1998) was considered to be the sign of diabetes. After that, animals were euthanised by a lethal dose of anesthetic.

2.2. Preparation of microvessels

Segments of the main intramuscular arteriole (1A, active diameter approximately 70–80 μm) were dissected from the cremaster muscle of age-matched control and streptozotocin (60 mg/kg, i.p.)-treated diabetic rats (2, 4 and 8 weeks of diabetes). The segments were then mounted in a custom-designed tissue chamber-perfusion system, in a bath containing Krebs' bicarbonate solution, oxygenation and pH 7.4 equilibrated with gas mixture of 5% CO_2 and 95% N_2 (Hill and Ege, 1994), by cannulating the two ends of the vessel onto glass micropipettes and securing with 0.2 nylon monofilament sutures (Alcon Surgical, USA), on the stage of an inverted microscope (Model IM35, Carl Zeiss) coupled to a high-resolution video system (Model WV-BP312, Panasonic, Japan). Then the micropipettes were connected to a pressure pump system (Living Systems, USA), the vessel was set as closely as possible to its in situ length, pressurised to in vivo levels (70 mmHg) and allowed to equilibrate at the maintained temperature of 33–34°C. Intraluminal pressure was monitored continuously by means of a pressure transducer (DPT-6000 Single-Use Transducer, Peter von Berg Medizinteknik, Englhartin, Germany). Preparations with leaks, apparent from the declining intraluminal pressure, were excluded from further study. Measurement of internal vessel diameter was accomplished using a manually adjusted electronic video caliper (Goodman, 1988).

2.3. Experimental protocol 1

In the first series of experiments, we investigated the responsiveness of the microvessels, at different stages of diabetes (control, 2, 4 and 8 weeks, $n = 5$ in each group), by using pharmacological (acetylcholine, an endothelial-dependent vasodilator; sodium nitroprusside, a nitric oxide donor and endothelial-independent vasodilator) and mechanical (pressure) stimuli.

Initially, the stable response of the vessel to a series of hydrostatic pressures was studied and randomized pressure–diameter relationships (in the range of 10–130 mmHg) were constructed. After returning to control level (70 mmHg), a concentration–response curve to acetylcholine (10^{-10}–10^{-5} mol/l) was constructed by allowing the diameter of the vessel to equilibrate at each concentration for approximately 5 min. After completion of the

acetylcholine curve, the tissue chamber was washed thoroughly until the vessel resumed control diameter. The response of the vessel to sodium nitroprusside was then studied using increasing concentrations (10^{-10}–10^{-6} mol/l) and a concentration–response curve constructed. At completion of each experiment, passive pressure–diameter relationships (10–130 mmHg) were determined after superfusion with a 0 Ca^{2+} Krebs-solution + 2 mM EGTA.

2.4. Experimental protocol 2

In the second series of experiments pathways mediating microvascular responses to chemical stimuli (acetylcholine) were investigated using pharmacological tools (*N*ω-nitro-L-arginine methyl ester (L-NAME), a selective NO synthesis inhibitor (NOS); indomethacin, a cyclooxygenase inhibitor) in similar groups of animals as characterized above.

First, a randomized active pressure–diameter curve was obtained, followed by an acetylcholine concentration–response curve, as previously described in Protocol 1. After that, the diameter returned to control level, L-NAME (10^{-4} mol/l) was added to the chamber and the vessel was allowed to equilibrate for 15 min. Then, a second acetylcholine concentration–response curve was completed to characterize the NOS-dependent component. An additional washout period was performed for 20–25 min in an attempt to eliminate all acetylcholine effect on the vessel. Subsequently, indomethacin (5×10^{-5} mol/l) was administered to the bath, and the vessel left for a further 15 min. Subsequently, a third acetylcholine concentration–response was performed on the vessel to reveal the extent of involvement of prostaglandins in the responses. Finally, the passive pressure–diameter relationship was acquired by changing the bath solution in the tissue chamber to a solution containing 0 Ca^{2+} + EGTA.

2.5. Solutions and drugs

Isolated vessel dissection buffer contained a solution of (in mM) 145 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 1.0 $MgSO_4$, 1.0 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 Na_2EDTA and 3.0 3-(*N*-morpholino) propanesulfonic acid (MOPS)-buffered physiological salt solution (PSS) (pH 7.4 ± 0.3) as well as 1% albumin. The osmolality of the solutions ranged between 290 and 310 mosM/kg H_2O.

In all experiments, the tissue-chamber and the vessel was filled with a modified Krebs buffer solution containing (in mM): 111 NaCl, 11.5 glucose, 25.7 $NaHCO_3$, 10 HEPES, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 4.9 KCl. The osmolality of the solutions ranged between 285 and 305 mosM/kg H_2O.

The modified Krebs buffer solution used to determine passive pressure diameter conditions was similar to the regular mixture except $CaCl_2$ was excluded and 2 mM EGTA added.

The following drugs were used in the experiments: pentobarbital (Abbot Australasia, Kurnell, Australia), streptozotocin (Sigma, St. Louis, USA), acetylcholine (Sigma), sodium nitroprusside (Sigma), *N*ω-nitro-L-arginine methyl ester (Sigma), indomethacin (Sigma).

2.6. Statistics

Statistical analyses were performed using sign test or Wilcoxon's signed rank test when comparing paired observations. When comparing non-paired observations Mann–Whitney *U*-test was used. To ascertain the significance between groups, we performed ANOVA statistical analyses in addition to the previous tests. Differences resulting in P values of 0.05 or less were considered significant. Values are given as Mean ± S.E.

3. Results

Time course and/or dose–response relationships of the recorded variables are illustrated in figures. Controls were carried out throughout the 8 weeks of the experiment studied. Data from the first five controls (weeks 6–9) and the last five controls (weeks 9–14) did not show any significant difference with respect to active or passive vessel diameter or response to acetylcholine (ANOVA, $P > 0.05$ for all categories). The stability of these measurements during this period is similar to that reported by other workers on rat skeletal muscle arterioles (Linderman and Boegehold, 1999). These authors also found stability of vessel response to NO and the application of L-NAME during this time period. Accordingly, all our control data was averaged for comparison with the 2, 4 and 8 weeks diabetic data. The statistical analyses were performed on results given in text.

3.1. Arteriolar diameter responses to randomized changes in intraluminal pressure

Active and passive pressure–diameter relationships (10–130 mmHg) during the progress of induced diabetes (control, 2, 4 and 8 weeks, $n = 10$ in each group) are illustrated in Fig. 1(A) and (B).

Randomly presented changes in intraluminal pressure resulted in normal development of myogenic responses in control vessels. Active resting diameters were not significantly different from controls at 2, 4 or 8 weeks diabetes for intraluminal pressures of 70 mmHg and above (control 67.5 ± 1.9 μm; 2 weeks diabetes 71.1 ± 2 μm, 4 weeks diabetes 62.4 ± 4.2 μm; 8 weeks diabetes 72.9 ± 3.7 μm; 70 mmHg; $n = 10$, $P > 0.05$). On the other hand, the active pressure–diameter relationship shifted character at 4 weeks diabetes showing a constriction of vessels at pressure 50 mmHg compared to controls consistent with increased rigidity and altered myogenic response (Fig. 1(A)). At 8 weeks of diabetes, vessels exhibited a significant

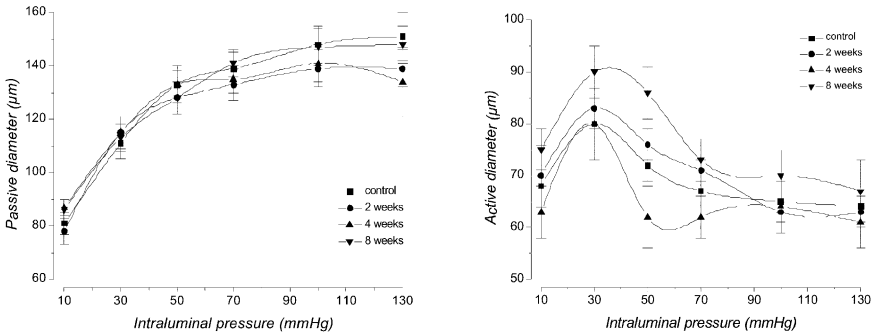

Fig. 1. (A) Intraluminal pressure–diameter active relationships of 1A cremaster arterioles in the presence of calcium at different stages (control, 2, 4 and 8 weeks) of streptozotocin induced diabetes; $n = 10$, means ± S.E.M. (B) Passive relationship, in the absence of calcium, between intraluminal pressure and vessel diameter in rat cremaster arterioles (1A) at different stages of experimental diabetes (control, 2, 4 and 8 weeks), $n = 10$. Means ± S.E.M.

dilation at 50 mmHg related to controls (8 weeks 85.8 ± 4.9 μm, controls 72.1 ± 2.8 μm, $n = 10$, $P < 0.05$). The shape of the passive pressure–diameter relationship remained unchanged showing no significant differences compared to control throughout all the stages of diabetes studied (Fig. 1(B)).

3.2. Altered vessel diameter responses to acetylcholine in diabetic animals

The effect of acetylcholine, an endothelium-dependent vasodilator (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} mol/l) on vessel diameter, administered into the vessel chamber, was studied at different ages of diabetes (control, 2, 4 and 8 weeks). At higher concentrations, acetylcholine was able to dilate control vessels to their maximum (passive) size. To quantify this, we introduced the percent of working range (PWR) at 70 mmHg: peak effect of the drug (μm) − active diameter (μm)/passive diameter (μm) − active diameter (μm). The maximum PWR value decreased significantly at 2 and 4 weeks of diabetes, but recovered to control values (i.e. maximum vessel dilatation) by 8 weeks of diabetes (percent of working range 106 ± 7.6% in control, 98.5 ± 3.5% in 8 weeks at 70 mmHg, $P > 0.05$, Fig. 2).

The sensitivity of the vessels to acetylcholine, indicated by the EC50 values, showed a decreasing trend with increasing duration of diabetes. The EC50 value at 8 weeks of diabetes was significantly less than that for the control preparations (control 4.22×10^{-8}; 8 weeks diabetes, 1.94×10^{-7} mol/l, $P < 0.005$).

3.3. Relationship between the effects of acetylcholine and sodium nitroprusside on vessel diameter

The endothelium-dependent vasodilator, acetylcholine, did dilate the vessel to its maximal diameter after an initial drop in peak responses at 2 and 4 weeks diabetes. On the other hand, sodium nitroprusside (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 5×10^{-7}, 10^{-6} mol/l), an endothelium-independent vasodilator failed to achieve similar effect on the

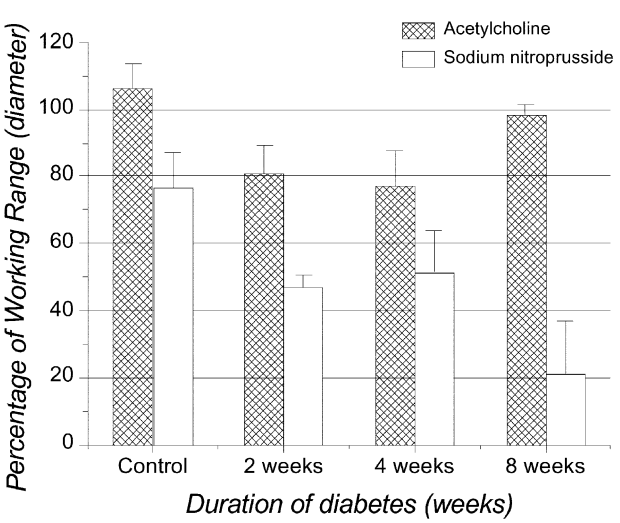

Fig. 2. The peak effect of acetylcholine and sodium nitroprusside on vessel diameter at 70 mmHg as expressed in percent of working range in various ages of diabetes. The calculation of percent of working range per se in text paragraph 3.2, $n = 5$–9. Means ± S.E.M.

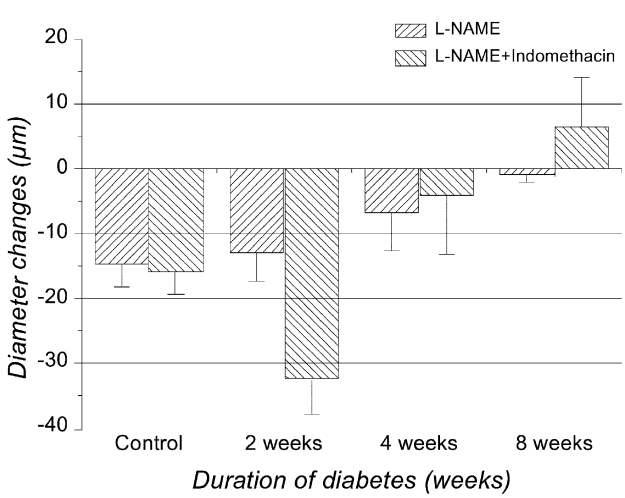

Fig. 3. Changes in baseline arteriolar diameter at 70 mmHg, 15 min after administering L-NAME (10^{-4} mol/l) and indomethacin (5×10^{-5} mol/l) into the bath containing vessels from control or diabetic rats (2, 4 and 8 weeks), $n = 5$. Means ± S.E.M.

microvessel diameter during the progress of diabetes. The effect of sodium nitroprusside was significantly reduced (compared to controls) at 2 and 8 weeks of diabetes. While acetylcholine could maintain the peak dilatation at 8 weeks of diabetes (PWR $98.5 \pm 3.5\%$), the effect of sodium nitroprusside (10^{-6} mol/l) was markedly reduced (PWR $20.9 \pm 15.9\%$ $P < 0.05$, Fig. 2).

3.4. The effect of L-NAME or L-NAME plus indomethacin on resting vessel diameter

For control animals the administration of L-NAME (10^{-4} mol/l) reduced the baseline diameter (at an intraluminal pressure of 70 mmHg) (Fig. 3). This effect gradually disappeared with the progress of diabetes (from -14.6 ± 3.4 μm (control) to -1 ± 1 μm (8 weeks diabetes), $P < 0.05$, Fig. 3).

Similarly to the above, a new resting diameter was recorded after administration of indomethacin (5×10^{-5} mol/l) (Fig. 3). The effect of L-NAME plus indomethacin was not significantly different from L-NAME alone for controls, 4 and 8 weeks diabetes. At 2 weeks diabetes the vessels showed a significant reduction in diameter compared to the effect of L-NAME alone (2 weeks, L-NAME —12.8 ± 4.6 μm; L-NAME + indomethacin—32.4 ± 5.3 μm, $n = 5$ per group, $P < 0.05$, Fig. 3).

3.5. The effect of L-NAME and L-NAME plus indomethacin acetylcholine-induced vasodilatation

To study the composition of the acetylcholine response, we compared the initial acetylcholine dose response curve with that obtained after treatment of the preparation with L-NAME and also following the subsequent addition of indomethacin. Maximal responses following L-NAME and indomethacin were not significantly different to those obtained with L-NAME alone. Therefore, the data presented is only for peak responses following administration of both L-NAME and indomethacin. In control preparations, a significant reduction in acetylcholine response was observed after administration of L-NAME together with indomethacin. (Fig. 4). At 2 weeks diabetes, a slight increase in response to acetylcholine was recorded. By 4 and 8 weeks diabetes, the response after L-NAME plus indomethacin was significantly greater than that for controls (control $-3.8 \pm 3.8\%$; 4 weeks diabetes $29.2 \pm 8.5\%$; 8 weeks diabetes $22.8 \pm 10.6\%$; $n = 5$, $P < 0.05$ for 4 and 8 weeks of diabetes compared to controls).

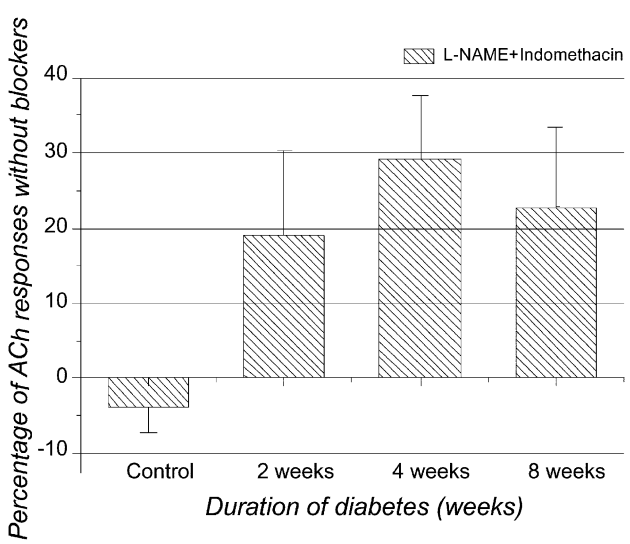

Fig. 4. Percentage of acetylcholine responses without blockers after adding L-NAME (10^{-4} mol/l) and indomethacin (5×10^{-5} mol/l) to the chamber solution. Animals used: control ($n = 4$), 2, 4 and 8 weeks diabetic, $n = 5$. Means ± S.E.M.

4. Discussion

This study aimed to follow the alteration of pathways mediating microvascular responses during the progress of streptozotocin-induced experimental diabetes.

4.1. Active and passive responses of arterioles to changes in pressure during experimental diabetes

Passive responses of diabetic vessels were not significantly different to those observed in control preparations. Under our experimental conditions, it is clear that this mechanical property of the vessel was remaining stable. Myogenic responses of the diabetic vessels were similar to controls at test pressures of 70 mmHg and above. However, the 4 weeks diabetic curve showed a significant constriction at 50 mmHg. Similar results were obtained by Hill and Ege (1994) in animals that had been diabetic for between 4 and 6 weeks. They suggested that changes in myogenic response might result from enhanced mechanical stiffness of the vessel. On the other hand, our observation suggests that this period of diabetes is associated with reduced effectiveness of tonic NO release and this could indicate that a lack of endothelial-dependent dilating influence might contribute to the overall level of constriction of the vessel. The loss in tone at 50 mmHg seen later in the diabetic process (8 weeks) could also be due to changes in the properties of the smooth muscle itself. Yu et al. (1996) have reported a decrease in vascular smooth muscle Ca^{2+} in older diabetic animals. Changes in both endothelial function and smooth muscle physiology may contribute to the alteration of the myogenic response seen during experimental diabetes.

4.2. Changes in effectiveness of nitric oxide pathway during the course of experimental diabetes

A reduction in the effectiveness of the NO pathway to cause dilation of the arteriole was observed under three different conditions.

Pro primo, with an intraluminal pressure held at 70 mmHg, the control preparations showed a notable dilating

effect to NO. The 'resting' modulation of the vascular smooth muscle has been reported by a number of authors for the isolated rat cremaster vessels. Endothelial removal in these preparations significantly reduced control diameter and treatment of such vessels with blockers of nitric oxide synthesis markedly attenuated control diameters suggesting a tonic vasodilator influence of the endothelium (Messina et al., 1992; Sun et al., 1992). Also, there is an indication for a regulatory feedback mechanism from smooth muscle via increased intracellular calcium level through myoendothelial cell junctions stimulating the tonic endothelial NO release (Dora et al., 1997), which is attenuated in diabetic animals causing a vasoconstriction (Zimmermann et al., 1997). Furthermore, supporting electrophysiological evidence of endothelial involvement has also been obtained from pressurised small mesenteric rat arteries. Application of L-NAME to this preparation decreased the membrane conductance and depolarised the vascular smooth muscle, implying a tonic release of NO by the endothelium (Weidelt et al., 1997). Koller et al. (1993) reported a maximum reduction in baseline diameter of 19% following treatment with *N*ω-nitro-L-arginine (L-NNA). This compares with our observations of 23% baseline diameter reduction in control vessels. This significant effect, however, diminished during the development of diabetes becoming abolished at 8 weeks diabetes. Despite the loss of effectiveness of tonic NO, the resting vessel diameter remained constant at 2, 4 and 8 weeks of diabetes compared to controls. It is apparent from above that stabilizing factors are present to offset the loss of dilatory influence (see below).

Pro secundo, the loss of effectiveness of the NO pathway is partly based on those experiments where sodium nitroprusside was added to the preparation. In control preparations, sodium nitroprusside was able to dilate the vessel up to about 80% of its working range. However, this effect diminished during the progress of diabetes reaching only about 20% at 8 weeks of diabetes. Although a reduction in the mechanical ability of cremaster vessels to dilate has been reported during experimental diabetes (Hill and Ege, 1994), this cannot account for our results for two reasons. Firstly, the passive diameter of the vessels did not vary significantly during the course of diabetes. Secondly, the vessels were still able to dilate maximally to acetylcholine after 8 weeks of diabetes, indicating that the endothelium-dependent vascular smooth muscle relaxation was not compromised.

Pro tertio, the reduced NO contribution to vessel diameter is supported by those experiments where acetylcholine was used to trigger dilation and L-NAME to selectively block the NO part of the stimulated response. In control preparation, the response could mostly be ascribed to NO activity. Moreover, other groups have reported a significant contribution of NO to the acetylcholine stimulated dilation of isolated vessels in the rat cremaster preparation (Sun et al., 1992; Koller et al., 1993). Bakker and Sipkema (1997) have also observed the NO-dependent steady state responses to acetylcholine in isolated rat cremaster resistance arteries. During the development of diabetes, we found that the contribution of NO to acetylcholine response declined as shown by the constantly increasing dilation after administering L-NAME into the tissue chamber.

4.3. Changes in the prostaglandin pathway during experimental diabetes

The activity of the prostaglandin pathways was more uncertain than that for the NO pathway. For the control preparations and 2, 4 and 8 weeks of diabetes, the prostaglandin pathway did not appear to be influencing the resting diameter of the vessel. However, at 2 weeks diabetes, the prostaglandins did exert some dilating influence on the resting vessel diameter based on a further reduction in vessel diameter after administration of indomethacin. Other workers have found the acetylcholine response of isolated rat cremaster arterioles to be resistant to the prostaglandin blocking effect of indomethacin (Messina et al., 1992). Our results are in line with those observations for control vessels. Even after 8 weeks of diabetes, the acetylcholine response did not show any significant contribution of prostaglandins. It is rather difficult to conclude that prostaglandin's are not active under these situations because the effect of dilator and constrictor prostaglandins could balance each other, thus to acetylcholine giving a conspicuous zero effect. Previous work has indicated that the endothelium in isolated rat cremaster vessels does have the capability to vary its production of dilator prostaglandins under different conditions (no flow), for example, during hypoxia or hyperoxia (Messina et al., 1992, 1994). However, the changes in prostaglandin activity or balance seen in our diabetic vessels remain to be elucidated. In addition, we must take into account that in our preparation, indomethacin was used in addition to L-NAME, i.e. after previous blocking of NOS activity. Nitric oxide may have a permissive effect on endothelial arachidonic acid metabolism (Bakker and Sipkema, 1998) and loss of NO in our preparation may, therefore, have affected the extent of prostaglandin activity.

4.4. Recovery and stability during experimental diabetes

Damage of the endothelium during the course of experimental diabetes has been widely reported in the literature. However, the results of our experiments suggest that although changes are occurring at and below the level of endothelium, there is a tendency towards recovery and stability of the system. Stabilisation of resting vessel diameter and stabilisation of the ability of the vessel to dilate maximally to acetylcholine appear to occur even in the face of reducing dependence of the system on the NO pathway.

For the resting diameter, the loss of tonic NO release is not accompanied by an expected constriction of the vessel (at 70 mmHg). As tonic release of NO and prostaglandins does not appear to be significant, it does remain a possibility that non-NO, non-prostaglandin factors are involved in the stabilisation of resting vessel diameter. A significant contribution of, e.g. endothelium-derived hyperpolarising factor (EDHF) to resting basal tone has been suggested by a study of hamster skeletal muscle arterioles in conscious animals (De Wit et al., 1999).

Maximal dilation of the vessel to acetylcholine recovers at 8 weeks of diabetes despite the loss of NO effectiveness. This recovery of acetylcholine response may be attributed, in part, to the presence of non-NO, non-prostaglandin factors. These factors provide about 23% of the response at 8 weeks of diabetes. Control preparations of isolated rat cremaster arterioles can show a significant contribution of a non-NO, non-prostaglandin factor (e.g. EDHF) to the initial transient response to acetylcholine although this component does not appear to contribute to the sustained response (Bakker and Sipkema, 1997). The importance of EDHF appears to increase as vessel size lowers concurrent with a decreasing NO role (Shimokawa et al., 1996). The contribution of EDHF to endothelium-dependent relaxation is significantly larger in microvessels than in large arteries in humans (Urakami-Harasawa et al., 1997). The same shift in balance between NO and EDHF, related to size, occurs in response to shear stress in mesenteric arteries (Takamura et al., 1999). Our experiments support the idea of a flexible transformation between NO and non-NO, non-prostaglandin-mediated dilation during the course of experimental diabetes studying one class of vessel. There is also evidence for a compensatory production of, e.g. EDHF to maintain acetylcholine-induced relaxation in hypertensive rat mesenteric vessels after chronic administration of L-NAME (Maeso et al., 1999). The relationship between the NO pathway and non-NO, non-prostaglandin pathways still needs to be clarified, but the evidence from in vivo hamster cremaster preparations suggests an inhibitory effect of NO on EDHF activity. In the presence of NO, the EDHF component of acetylcholine-induced dilation was reduced (De Wit et al., 1998).

In summary, we can conclude that there is a recovery of acetylcholine responses in arterioles during streptozotocin-induced diabetes and stabilisation of resting vessel diameter despite the loss of effectiveness of nitric oxide. These changes may reflect the plasticity of alternative pathways (e.g. non-NO, non-prostaglandin pathways) as a mechanism to compensate for the impact of diabetes on the vasculature.

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