Temporal changes in vascular reactivity in early diabetes mellitus in rats: role of changes in endothelial factors and in phosphodiesterase activity

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Submitted 29 January 2009; accepted in final form 16 June 2009

Abboud K, Bassila J, Ghali-Ghoul R, Sabra R. Temporal changes in vascular reactivity in early diabetes mellitus in rats: role of changes in endothelial factors and in phosphodiesterase activity. Am J Physiol Heart Circ Physiol 297: H836–H845, 2009. First published June 19, 2009; doi:10.1152/ajpheart.00102.2009.—The aims of this study were to study the influence of the duration of diabetes, the role of endothelial-derived vasodilators, and the role of phosphodiesterase (PDE) isoform activity in the early changes in vascular reactivity of aortic rings from diabetic rats. Diabetes mellitus was induced in female rats by intravenous streptozotocin (85 mg/kg). Two or 4 wk later, thoracic aortic rings from control and diabetic rats were isolated, and vascular responses to acetylcholine (ACh), S-nitroso-N-acetylpenicillamine (SNAP) [nitric oxide (NO) donor], DMPPO (PDE5 inhibitor), and phenylephrine (PE) were obtained in the presence and absence of endothelium or other drugs. PDE isoform activity was also measured. At 2 wk, responses to ACh and DMPPO were enhanced, whereas those to PE were attenuated in diabetic rats relative to controls. Indomethacin and SQ-29548 (a thromboxane A2 receptor antagonist), but not Nω-nitro-L-arginine methyl ester, corrected these differences. The responses to SNAP, and cAMP and cGMP hydrolytic activities, were similar in the two groups. In contrast, at 4 wk, ACh, DMPPO, and PE produced similar responses in the two groups: Nω-nitro-L-arginine methyl ester rendered the response to PE lower in the diabetic group, and this was corrected by indomethacin, but not SQ-29548, treatment. The response to SNAP was greater in the diabetic group, and this was corrected by DMPPO. Activity of all PDEs was decreased at 4 wk. We conclude that, at 2 wk, there is modulation of thromboxane A2 production, but no change in the NO system or PDE isoform activities. At 4 wk, a reduction in NO activity is superimposed; at this stage, PDE activity is reduced, together with increased production of vasodilating prostaglandins, possibly as a compensatory mechanism to maintain normal vascular reactivity. 

endothelium; vasodilation; vasoconstriction

VASCULAR COMPLICATIONS ARE an important cause of increased mortality in patients with diabetes mellitus (8). Vascular dysfunction in diabetes has been linked to alterations in the function of the endothelium, and endothelial dysfunction itself has been linked to development of atherosclerosis (7, 33).

Extensive studies have been conducted in various animal models of both type I and type II diabetes mellitus to explore the changes in vascular function and the mechanisms underlying them. These studies demonstrated alterations in vascular responses to both vasodilators and vasoconstrictors. Decreased endothelium-dependent relaxation has been observed in blood vessels from several models of diabetes in rats and in humans (13, 22, 23, 30). In contrast, enhanced endothelium-dependent relaxation was reported in several other studies (3, 29, 41). Conflicting results were also obtained when responses to vasoconstrictor agents were examined, whereby some studies reported impaired contractile responses to vasoconstricting agents in streptozotocin (STZ)-induced diabetic rats (32, 45), spontaneously diabetic rats (17), and alloxan-induced diabetic rabbits (436), while others reported an enhanced vascular reactivity to vasoconstricting agents (39, 41). To further complicate the matter, a number of studies reported no changes in vascular reactivity to endothelium-dependent vasodilators (11, 25), endothelial-independent vasodilators (11, 25, 39), and vasoconstrictors (17, 39).

In line with these observations, conflicting results have been obtained from studies that examined the underlying defect in endothelial function leading to these altered vascular responses in diabetes. Some investigators suggested a decrease in nitric oxide (NO) activity (1, 27), while others proposed an impairment in endothelium-derived hyperpolarizing factor (EDHF) activity (39, 42) or in cyclooxygenase activity (4, 38) as causes of the significant decrease in endothelium-dependent vasodilation in diabetic tissues. In contrast, other studies reported an enhanced endothelium-dependent relaxation secondary to increased production of NO (2, 26, 35) or prostacyclin (2, 9, 14, 18, 28).

It is clear that the results of vascular reactivity studies in animal models of diabetes are contradictory, and there is not an entirely satisfactory explanation for the variable responses of diabetic blood vessels to vasoconstrictor and vasodilator drugs. Possible explanations may involve differences in species, duration of diabetes, etiology of diabetes, type of vascular preparation studied, and type of vasoconstrictor or vasodilator used in the various studies, among other factors. In this context, Pieper (31) suggested that the duration of diabetes was an important factor, showing that the responses of aortic rings to acetylcholine (ACh) were enhanced 1 day after STZ injection, unchanged at 1–2 wk, and reduced at 8 wk, and ascribed these differences to changes in NO production. Similarly, Zhu et al. (44) found that responses to phenylephrine (PE) were increased at 2 and 6 wk, but reduced at 12 wk, but did not explore the underlying mechanisms.

While much attention has been directed at the production of endothelial vasodilators, few studies have investigated the role of changes in phosphodiesterase (PDE) activity in modulating vascular function. The biological effect of NO and prostaglandins are transduced by cGMP and cAMP, respectively, cyclic nucleotides that are hydrolyzed by different PDEs. A change in PDE activity in diabetes can thus influence the levels of these compounds and modify the effects of these endothelial mediators. In a recent study, our laboratory showed that, in an animal model of liver cirrhosis, which is characterized by peripheral vasodilation, decreased efficacy of vasoconstrictors,
and enhanced endothelial-dependent vasodilation, there was evidence suggesting that changes in PDE5 activity may contribute to the observed changes in vascular reactivity, by decreasing the hydrolysis of cGMP (37). Few studies have looked at regulation of PDE activity in diabetes mellitus and their contribution to the observed changes. Miller et al. (24) noted a decrease in cGMP and cAMP hydrolysis in the penile and aortic tissue of 2-mo diabetic rats, but did not conduct functional studies, whereas Matsumoto et al. (20) found increased expression of PDE3 isoforms (but not PDE4) in mesenteric arteries of 12-wk diabetic rats, as well as enhanced responses to the EDHF component of ACh vasodilation; the latter was corrected by cilostazol, an inhibitor of PDE3.

The purpose of this study was to use the model of STZ-induced diabetes mellitus in the rat to 1) systematically examine the responses of aortic rings to vasoconstrictors and endothelium-dependent and -independent vasodilators at two time points early in the course of diabetes mellitus in rats (at 2 and 4 wk after induction); 2) examine systematically the contribution of endothelial factors (NO, eicosanoids, and EDHF) in these altered responses; and 3) examine the changes in activity of PDE isoforms (PDE1–5) and the role of PDE5 in the observed changes in vascular function in diabetes mellitus.

**MATERIALS AND METHODS**

This study was approved by the Institutional Animal Care and Use Committee of the American University of Beirut.

Female Sprague-Dawley rats weighing 180–220 g were divided into two groups. Insulin-dependent diabetes mellitus was induced in the first group by injection of 85 mg/kg STZ dissolved in citrate buffer solution into the tail vein under ether anesthesia. The control group was injected with the citrate buffer solution not containing STZ.

Diabetic and control groups of rats were further divided into two subgroups. The first subgroup of rats was used for studies of aortic vascular responses and PDE activity at 2 wk after the above treatment, whereas the second subgroup was used after 4 wk. At the time of the study (2 or 4 wk), blood samples were collected from diabetic and control rats for measurement of blood glucose concentrations using a blood glucose meter (Accu-Chek sensor).

**Measurement of Vascular Responses**

At the end of each period, the rats were killed by decapitation, and their thoracic aortas were removed and immediately placed in modified Krebs-Henseleit solution composed of the following (mmol/l): 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.17 MgSO4, 1.18 KH2PO4, 25.0 NaHCO3, 0.026 EDTA, and 11.1 d-glucose. The vessels were cleaned of connective tissue and cut into four rings, each of 2-mm length. In some preparations, the endothelium was removed by gently rubbing the intimal surface of the vessel with a glass rod.

Endothelium-intact or denuded rings from all groups of rats were placed in individual organ chambers, each filled with 15 ml of modified Krebs-Henseleit solution and kept at 37°C all through the experiment, with an outer water jacket and a circulating heat pump. Each chamber was also continuously bubbled with a mixture of 95% O2 and 5% CO2. The rings were attached to a force-displacement transducer. Tensions were recorded on a multichannel polygraph (Gould 1302–1306, World Precision Instruments) and expressed as absolute values (g).

The vessels were allowed to equilibrate for 1 h and were washed with the above-mentioned solution before starting the experiments. A baseline force of 2.0 g was applied to the rings. Once the ring tension had stabilized, each ring was induced to contract with 10–6 M of PE (α1-adrenoreceptor agonist). The presence of functional endothelium was determined on the basis of relaxation evoked by 10–5 M ACh. Rings showing ≥60% relaxation of PE contraction were considered as containing endothelium.

After testing for endothelium, the rings were washed three times with warm modified Krebs-Henseleit solution, and, once the tension had stabilized, they were used to run one of the following protocols in both control and diabetic rats (each ring was used to run one experiment only).

1) Aortic rings with endothelium were precontracted with 10–6 M PE, and cumulative concentration-response curves were obtained in control and diabetic rings to ACh (10–9 to 10–5 M) or to the specific PDE5 inhibitor DMPO (1,3-dimethyl-6-(2-propoxy-5-methane sulphonylaminophenyl)-pyrazolo[3,4-d]pyrimidin-4(5H)-one), at concentrations ranging from 10–9 to 10–5 M (6).

2) Aortic rings denuded of endothelium were exposed to increasing concentrations of SNAP (S-nitroso-N-acetylpenicillamine), a NO donor that does not require enzymatic activity to release NO (12), at concentration ranging from 5 × 10–8 to 5 × 10–6 M until maximum effect was achieved.

In many cases, the above experiments were conducted after incubation with one of the following drugs for the time indicated (these experiments were run in different rings, not repeated on the same rings described above): 1) DMPO (10–7 M) to inhibit PDE5 activity, for 10 min; 2) Nω-nitro-l-arginine methyl ester (l-NAME; 0.3 mmol/l) to inhibit NO activity, for 10 min; 3) tetraethylammonium (TEA; 1 × 10–3 M), a nonselective Ca2+–activated K+ channel inhibitor, to inhibit EDHF-like activity, for 10 min (it should be noted, however, that, since the nature of EDHF is not fully defined and may indeed be more than one substance or mechanism, this intervention may not fully block that component of vasodilatation attributed to EDHF activity; 4) indomethacin (10–5 M) to inhibit cyclooxygenase activity, for 20 min; and 5) SQ-29548 (10–6 M), a thromboxane (Tx) A2 receptor antagonist, for 15 min.

Each rat was considered as one experiment, even when more than one ring was used for the same protocol. In such cases, the average response from two or more rings was taken and used to represent the rat’s response. For experiments involving PE, the response was considered as the difference between absolute tension developed and baseline tension. For vasorelaxants, the response was depicted as the percent relaxation relative to the tension developed in response to PE.

**Measurement of PDE Activity**

At 2 or 4 wk following administration of STZ or its vehicle, rats were killed by decapitation under CO2 narcosis, and the thoracic aorta and the superior mesenteric artery (to the level of mesenteric arterioles) were isolated, cleaned of adjacent tissues, immediately frozen in liquid N2, and stored at −80°C. At the time of the assay, the frozen tissues were ground in liquid N2. The resulting tissue powders were homogenized with a glass Potter for 3 × 10 s at 4°C, with 100 mg/ml of the following buffer: 2 mM magnesium acetate, 5 mM EGTA, 1 mM dithiothreitol, 10 μg/ml leupeptin, 10 μg/ml aprotinin (Trasylol), 10 μg/ml chymostatin, 10 μg/ml pepstatin, and 10 μg/ml antipain.

**Table 1. Plasma glucose concentrations 2 and 4 wk after injection of streptozotocin or its vehicle (citrate buffer) at the time the vascular reactivity experiments were conducted**

<table>
<thead>
<tr>
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<th>Control</th>
<th>Diabetic</th>
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<tr>
<td>2 wk</td>
<td>141 ± 16</td>
<td>516 ± 22*</td>
</tr>
<tr>
<td>4 wk</td>
<td>145 ± 13</td>
<td>488 ± 46*</td>
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Values are means ± SE of glucose measurements in mg/dl. *P < 0.05, diabetic vs. control.
soya trypsin inhibitor, 2,000 U/ml aprotinin, 0.33 mM Pefabloc, and 20 mM Tris, pH 7.5. The homogenates were divided into small aliquots and stored at \(-80^\circ C\) until used. Protein concentration was determined following Lowry et al. (19).

Total PDE activity was determined at 1 \(\mu M\) cAMP or cGMP in the presence of 1 mM EGTA by a radioenzymatic assay, as previously described (16). To determine the distinct PDE isozyme contribution in the overall cyclic nucleotide hydrolyzing activity, PDE family-specific inhibitors were included in the assay medium, as described elsewhere (15). The proportion of cGMP-hydrolyzing PDE isozymes in the homogenates was assessed at 1 \(\mu M\) cGMP in the presence of 10 \(\mu M\) nimodipine for PDE1, in the presence of 0.1 \(\mu M\) DMPPO for PDE5, and 20 \(\mu M\) EHNA for PDE2. The proportion of cAMP-hydrolyzing PDE isozymes was assessed at 1 \(\mu M\) cAMP in the presence of 1 mM EGTA, including 1 \(\mu M\) cilostamide for PDE3 and 10 \(\mu M\) rolipram for PDE4.

The results are expressed as means \(\pm\) SE of the average weight in g. *\(P < 0.05\), diabetic vs. age-matched controls.

### RESULTS

Rats treated with STZ sustained a marked rise in plasma glucose levels as expected (Table 1) and a significant loss of weight relative to control animals (Table 2).

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>2 wk</th>
<th>Baseline</th>
<th>4 wk</th>
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<tbody>
<tr>
<td>Control rats</td>
<td>189±2</td>
<td>223±3</td>
<td>192±2</td>
<td>252±3</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>189±2</td>
<td>180±4*</td>
<td>192±2</td>
<td>195±6*</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE of the average weight in g. *\(P < 0.05\), diabetic vs. age-matched controls.

Fig. 1. Effect of acetylcholine on phenylephrine (PE)-precontracted aortic rings from diabetic and control rats 2 wk after administration of streptozotocin (STZ; A), and after preincubation with \(N^G\)-nitro-L-arginine methyl ester (L-NAME; B), indomethacin (C), a combination of L-NAME and indomethacin (D), or SQ-29548 (E). Values are means \(\pm\) SE. *\(P < 0.05\), #\(P < 0.01\) compared with the value in the corresponding control rings.
Studies Conducted at 2 wk

Effects of ACh, SNAP, and DMPPO. ACh produced dose-dependent relaxation in PE-precontracted rings, which were significantly enhanced in rings from diabetic rats relative to controls (P < 0.0001, Fig. 1A). Incubation of the rings with L-NAME prevented the relaxant response to ACh, but there was still a difference between the two groups, with greater contraction in the control group (P < 0.0001, Fig. 1B). Indomethacin reversed the difference between diabetic and control rings (P < 0.03, Fig. 1C) and reduced this difference in the presence of L-NAME (P < 0.02, Fig. 1D). Close inspection showed that indomethacin significantly enhanced the response to ACh in the control group, but had no effect in the diabetic group. SQ-29548 corrected the difference between groups by enhancing the response in the control group (Fig. 1E).

In endothelium-denuded aortic rings, the responses to all concentrations of SNAP were similar in control and diabetic rats (Fig. 2). The vasorelaxant response to DMPPO, however, was significantly higher in rings from diabetic rats than in those from control rats at all concentrations used (P < 0.0001, Fig. 3A). Removal of the endothelium almost completely annulled the effect of DMPPO in both groups, although there remained a slight effect in the diabetic group, which was significantly different from that in the control group (P = 0.0064, Fig. 3B). In the presence of L-NAME, the vasodilator effect was reduced but not annulled, and the difference between the two groups was eliminated (Fig. 3C). Interestingly, indomethacin treatment reversed the difference in response to DMPPO between the two groups such that the response in the diabetic group became greater than that in the control group (P = 0.008, Fig. 3D). The same change was observed with SQ-29548 (P = 0.021, Fig. 3E).

Effects of PE. PE produced concentration-dependent contraction in thoracic aortas of diabetic and control rats. In the presence of endothelium, PE-induced contraction was significantly lower in diabetic compared with control rats (P < 0.0001, Fig. 4A). Removing the endothelium shifted the concentration-response curves to the left in both diabetic and control rats and markedly reduced the difference between them (P = 0.004, Fig. 4B). When the endothelium-rich rings from diabetic and control rats were preincubated with L-NAME, the concentration response curves of PE in both diabetic and control rats were shifted to the left significantly, and the maximal response was increased, but there was still a hyporesponsiveness in aortic rings from diabetic rats compared with control rats at all concentrations of PE (P < 0.0001, Fig. 4C).

Preincubation with TEA (1 × 10⁻³ M) also enhanced the response to PE in both diabetic and control rats; however, the response of aortic rings from diabetic rats remained significantly lower than that in rings from control rats (data not shown). Preincubating the vessels with a combination of TEA and L-NAME did not eliminate the difference in response to PE between diabetic and control rats (data not shown). In contrast, after incubation with indomethacin (10⁻⁵ M), there was no longer any significant difference between the concentration-response curves from diabetic or control rats in response to PE (Fig. 4D). Thus indomethacin eliminated the difference in the response to PE previously observed in diabetic and control rats, and this was due to a significant decrease in the response observed in the control group. Similar results were obtained when SQ-29548 was used instead of indomethacin (P = 0.0005, Fig. 4E).

Studies Conducted at 4 wk

Effects of ACh, SNAP, and DMPPO. In contrast to what was observed at 2 wk, the relaxant responses to ACh 4 wk after STZ became significantly lower in rings from diabetic rats compared with control rats (P = 0.0064, Fig. 5A). L-NAME almost completely abolished the vasodilator response to ACh, but there remained a difference between the two groups, with greater contraction in the control group (Fig. 5B). In the presence of indomethacin, however, the difference in vasodilator response to ACh became more evident and more significant (P < 0.0001, Fig. 5C). Incubation with SQ-29548, however, had a different effect compared with that of indomethacin and completely annulled the difference between the two groups (Fig. 5D).

SNAP produced concentration-dependent relaxation of denuded thoracic aortas previously contracted with PE from both diabetic and control rats. Also, in contrast to what was observed at 2 wk, the responses to SNAP were significantly greater in aortic rings from diabetic relative to control rats (P < 0.0001, Fig. 6A). Pretreatment with DMPPO enhanced the response in the control group and reversed the difference between diabetic and control rings in response to SNAP (P = 0.0008, Fig. 6B). Finally, the responses to DMPPO were also different from those observed at 2 wk in that the difference between the two groups was minimal (P = 0.034, Fig. 7).

Effects of PE. PE produced a concentration-dependent contraction of endothelium-intact aortic rings in both groups of rats. In contrast to what was observed at 2 wk, however, the response was similar in the two groups (Fig. 8A). As expected, removal of the endothelium enhanced the response to PE in both control and diabetic rats (Fig. 8B). Neither TEA nor indomethacin alone had any effect on the concentration-response relationship (data not shown). After incubating the rings with L-NAME, however, PE-induced contraction was observed to be significantly lower in the preparations isolated from diabetic rats compared with con-

![Fig. 2. Relaxation induced by S-nitroso-N-acetylpenicillamine (SNAP) in PE-precontracted aortic rings from diabetic and control rats 2 wk after STZ administration. Values are means ± SE.](http://ajpheart.physiology.org/Downloaded from http://ajpheart.physiology.org)
trol rats ($P = 0.0008$, Fig. 8C). This difference was annulled when indomethacin was added to the incubation medium (Fig. 8D). In contrast, the combination of SQ-29548 with L-NAME reduced the response to PE in both groups, but it did not eliminate the difference between them ($P < 0.0001$, Fig. 8E).

**PDE Activity**

After 2 wk of treatment with STZ or vehicle, there was no difference in total cAMP or cGMP hydrolytic activity in aortic rings from diabetic rats relative to those from control rats (Table 3). In contrast, after 4 wk of treatment, the situation was completely different, such that there was a decrease in total cAMP activity, associated with a decrease in PDE3 but not PDE4 activity, in aortic rings from diabetic rats relative to those from control rats (Table 3). Total cGMP hydrolytic activity and PDE1 and PDE5 activities were also significantly reduced in aortic rings from diabetic rats.

**DISCUSSION**

This study demonstrates that the impact of STZ-induced diabetes on the vascular response of thoracic aorta in rats varies with time, and that the nature of the endothelial dysfunction also changes over time. The results suggest a major role for changes in production of eicosanoids and/or NO, and in activity of PDE isoforms, in altering the responsiveness of aortic rings from diabetic rats at 2 and 4 wk after STZ injection. The significance and advantage of this study relative to most previous published reports are that 1) it looked at the unfolding changes in vascular function early in this model of diabetes, which has been used extensively in the literature with conflicting results; 2) it examined vascular function by considering responses to both vasodilators (endothelium dependent and independent) and vasoconstrictors at two different time points in the same study, using the same model of diabetes, the same vascular preparation, and the same experimental conditions and setup; thus the conclusions regarding the role of the various factors and the
influence of time should be more solid than those derived from studies conducted by different investigators under different conditions using either vasoconstrictors or vasodilators; and 3) it is the first to demonstrate a functional role for PDE isoforms, particularly PDE5, in the changes in vascular function induced by diabetes.

Rats with diabetes mellitus of 2-wk duration had an enhanced vasorelaxation in response to ACh. This enhancement was annulled by indomethacin, which suggested that diabetes was associated with increased production of vasodilating prostaglandins. However, indomethacin treatment did not alter the response in the diabetic group, but rather enhanced the response in the control group, suggesting that, at early stages of diabetes, there is a decrease in production of vasoconstricting prostaglandins, i.e., TxA2. This was confirmed by the effect of SQ-29548, which, like indomethacin, eliminated the difference between the two groups by enhancing the response to ACh in the control group. Interestingly, it was clear that use of l-NAME, while markedly inhibiting the vasodilator effect of ACh, did not eliminate the difference between vessels from control and diabetic rats, with the latter sustaining a significantly lower contraction in response to ACh (see Fig. 1B). In the absence of NO, it is likely that whatever effect is observed is due to a balance between endothelial-mediated effects and the direct vasoconstrictor effect of ACh on smooth muscles. Inhibiting the endothelial COX-related contribution by indomethacin corrected this difference, lending further support to our conclusion that, at 2 wk of diabetes, it is the cyclooxygenase rather than the NO system that is modulated.

The lack of difference in responses to SNAP suggests that PDE activity (specifically cGMP hydrolysis) is not affected at this stage of diabetes. This was confirmed by the measurement of cGMP hydrolysis, PDE1, and PDE5 activities. The increased response to DMPPO in diabetic rats, however, is not easily explained. DMPPO is supposed to be a PDE5-specific inhibitor; thus its relaxant effect should be mediated by enhancement of the effect of cGMP-generating...
Five possible explanations exist for the hyperresponsiveness to PE seen in 2-wk diabetic rats: 1) an increase in the contribution of EDHF; 2) an enhanced release of endothelium-derived NO; 3) an increase in the production of vasoconstrictor eicosanoids; 4) a decrease in the production of endothelial vasoconstrictor; and 5) a decrease in PDE5 activity. The last possibility can be eliminated based on the measurements reported in Table 3. The fact that neither L-NAME nor TEA were able to correct the difference between the two groups of aortic rings suggests that neither NO nor EDHF contribute to this hyperresponsiveness. Preincubating the vessels with indomethacin, however, eliminated the difference in PE response between diabetic and control rats, suggesting that the lower contractile response to PE is due to an enhanced effect of eicosanoids at 2 wk of diabetes. However, the correction of the difference was due to a decrease in the contractile effect of PE in the control group and not an enhancement of the effect in the diabetic group. This suggested that the control group had an excess of a vasoconstrictor prostaglandin (TxA2). The fact that

substances. The fact that indomethacin and SQ-29548 eliminated the difference between the two groups and that L-NAME did not completely prevent the vasodilator effect of DMPPO suggest that this compound is not as selective as previously thought, and that other mechanisms may contribute to the observed vasorelaxant effects. This renders the interpretation of the results quite problematic and prompts further investigation into the mechanisms of action of the drug.

The results of the experiments with PE-induced vasoconstriction confirm the above conclusions. Those results showed an impaired response to increasing concentrations of PE in endothelium-intact aortic rings from 2-wk diabetic rats. The contractile response to PE was significantly enhanced by removing the endothelium from the aortas in both STZ-diabetic and control rats, and there was no longer a difference in response between the two. This suggests that an endothelium-derived relaxing factor(s) may be attenuating the vascular contraction in response to PE.

Fig. 5. Effect of acetylcholine on PE-recontracted aortic rings from diabetic and control rats 4 wk after administration of STZ (A), in the presence of L-NAME (B), and in the presence of indomethacin (C) or SQ-29548 (D). Values are means ± SE. *P < 0.05, **P < 0.01, ***P < 0.001, compared with the value in the corresponding control rings.

Fig. 6. Relaxation induced by SNAP in PE-precontracted aortic rings from diabetic and control rats 4 wk after STZ administration in the absence (A) and presence (B) of DMPPO. Values are means ± SE. *P < 0.01, **P < 0.001, compared with control group receiving the same treatment.
SQ-29548 reproduced the effects of indomethacin supports this conclusion. These results and conclusions are consistent with what was observed with ACh.

In conclusion, STZ-induced diabetes of 2-wk duration in rats is characterized by a decreased production of TxA2, causing higher responses to ACh and lower responses to PE. We also suggest that, at this stage of the disease, neither the NO system nor the activities of the various PDEs are appreciably perturbed.

Diabetes mellitus of 4-wk duration, however, had a different impact on the aortic response to vasopressor and vasorelaxant agents. The responses to ACh became less in the diabetic group, and, in the presence of indomethacin, this difference was further accentuated. A possible explanation for this is that the production of NO by aortic rings from diabetic rats is reduced relative to controls, while that of vasodilating eicosanoids is increased, with the latter serving to maintain a normal response to ACh. Thus when prostaglandin production was inhibited by indomethacin, the deficiency in NO production became apparent. In the 4-wk diabetic group, SQ-29548 had an effect distinct from that of indomethacin, completely eliminating the difference.
between the two groups. This suggests a complex picture of decreased NO production and increased production of both vasodilating prostaglandins and TxAs (in contrast to the 2-wk model) in diabetic rats. In the presence of SQ-29548, the deficiency in NO is countered by an excess of vasodilating prostaglandins to maintain a response similar to control, whereas indomethacin, by eliminating the effect of both vasodilating and constricting prostaglandins, renders the response to ACh less in the diabetic animals, which are left with a deficiency of NO.

At 4 wk of diabetes, the responses to SNAP became enhanced in the diabetic group, which contrasts with the results at 2 wk. This suggested that cGMP hydrolytic activity was reduced in these rings, supported by the fact that DMPPO (inhibitor of PDE5) corrected the difference in response to SNAP. This conclusion was confirmed by the results demonstrating a reduction in total cGMP hydrolysis and PDE1 and PDE5 activities at 4 wk.

DMPPO-induced concentration-dependent relaxation of aortic segments from diabetic rats was not markedly different, at 4 wk, from that obtained in aortas from control rats. Our finding of increased endothelium-independent relaxation to SNAP and the finding of decreased PDE5 activity in the aortic tissue at this stage of the disease suggest that the response to DMPPO should be different. This true provided NO production is the same. Thus it is possible that, together with diminished PDE5 activity, a decrease in NO production exists at this stage of diabetes, as suggested by the ACh results. As such, the reduced cGMP hydrolysis could constitute a compensatory mechanism meant to counter the decreased NO production, to maintain the same NO signal. To further explore this possibility, we examine the responses to PE.

PE-induced contraction was similar in both endothelium-intact and endothelium-denuded aortic segments from 4-wk diabetic and control preparations, in contrast to what was observed in the 2-wk model. It would appear, therefore, that no alteration in vascular reactivity or endothelial function existed at this time. After preincubation with L-NAME, however, the response to increasing concentrations of PE was significantly lower in diabetic rats. This suggested that, after inhibiting NO production, an excess of a relaxing factor became apparent in diabetic preparations, and that this excess was masked by the fact that NO production was deficient in 4-wk diabetic rats. Pretreatment with indomethacin corrected the lower response to PE seen in L-NAME-treated rings, suggesting a role for vasodilating eicosanoids in this response. SQ-29548 reduced the responses to PE in both groups but, in contrast to indomethacin, did not eliminate the difference between them; this supports our conclusion that, at 4 wk, there is an increase in the production of both vasodilating and vasoconstricting prostaglandins in vessels of diabetic rats.

In conclusion, STZ-induced diabetes of 4-wk duration in rats is characterized by a reduction in NO synthesis, associated with concomitant reduction in cGMP and cAMP hydrolysis due to suppression of PDE activity, and a persistent activation of the cyclooxygenase pathway, with the latter three possibly acting as compensatory mechanisms for the decrease in NO production.

Consistent with our data, several investigators (2, 9, 18, 28) indicated that the enhanced ACh-relaxant potency in diabetes is caused by increased mediation of prostacyclin. Shen et al. (34) also suggested an increase in function and content of PGI2 contributing to the enhanced endothelium-dependent vasodilatation in STZ-induced diabetic mice, and they also presented a significant increase in PGI2 serum content from STZ-induced diabetic mice compared with age-matched controls. This is also consistent with studies demonstrating mediation by endogenous prostaglandins of the increases in gastric and mesenteric blood flow in diabetics (9), and increased renal production of prostacyclin in diabetic rats (28).

In addition, many studies in experimental animals designed to investigate the mechanism involved in endothelial dysfunction in diabetes have implicated decreased NO availability as the most important factor involved in such dysfunction (10, 21, 43). Kobayashi et al. (17) reported an impaired ACh-mediated relaxation and suggested that this was due to decreased NO production and responsiveness at late stages of spontaneous diabetes. The present study suggests that this decrease begins as early as 4 wk after diabetes mellitus (in the STZ model), but that, at this stage, the decrease in cGMP hydrolysis and the increase in vasodilating eicosanoids compensate for it.

In summary, this study shows that diabetes induces time-dependent alterations of endothelial function in aortic preparations from STZ-induced diabetic rats. At 2 wk, there appear to be a selective decrease in the activity of TXA2 and no change in NO production or PDE activity. In contrast, at 4 wk, NO production is reduced, and this is associated with decrease in PDE activity and an increase in cyclooxygenase products, including vasodilating prostaglandins, to compensate for the reduction in NO activity. The results help clarify the controversial data present in the literature and point to a new mechanism by which diabetes may influence vascular function, through altering PDE activity.

ACKNOWLEDGMENTS

The technical help of Rowayda Khattab and Nahed Mougharbel is greatly appreciated. We thank Dr. Claire Lugnier, Université Louis Pasteur, Strasbourg, and Dr. Rima Tahasledar, Lebanese University, for help in establishing the PDE assay, and Dr. Lugnier for providing DMPPO.

<table>
<thead>
<tr>
<th>Enzyme Activity, pmol·mg⁻¹·min⁻¹</th>
<th>2 wk</th>
<th>4 wk</th>
<th>2 wk</th>
<th>4 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cAMP hydrolytic activity</td>
<td>37.5±3.9</td>
<td>42.3±4.7</td>
<td>44.7±2.8</td>
<td>28.6±1.7†‡</td>
</tr>
<tr>
<td>PDE3</td>
<td>18.0±1.6</td>
<td>18.5±2.7</td>
<td>26.1±1.8</td>
<td>16.4±1.3‡‡</td>
</tr>
<tr>
<td>PDE4</td>
<td>14.8±1.9</td>
<td>15.1±2.8</td>
<td>12.2±2.3</td>
<td>8.2±1.1</td>
</tr>
<tr>
<td>Total cGMP hydrolytic activity</td>
<td>18.9±4.0</td>
<td>20.6±1.3</td>
<td>23.1±3.9</td>
<td>9.0±1.1†‡</td>
</tr>
<tr>
<td>PDE1</td>
<td>2.3±1.3</td>
<td>4.1±0.5</td>
<td>4.7±1</td>
<td>3.4±0.1*</td>
</tr>
<tr>
<td>PDE5</td>
<td>8.9±1.8</td>
<td>8.1±0.9</td>
<td>12.3±2.8</td>
<td>3.6±0.6†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. PDE, phosphodiesterase. *P < 0.05, †P < 0.01, ‡P < 0.001, compared with control.
GRANTS

This study was funded by grants from the Medical Practice Plan Research Fund of the Faculty of Medicine and from the American University of Beirut University Research Board.

REFERENCES