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Impairment of endothelium-dependent relaxation in aortae from spontaneously diabetic rats

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1 Diabetes mellitus is known to produce alterations in vascular reactivity. The present study examined the effects of endothelium-dependent and endothelium-independent relaxing substances on thoracic aorta from control and spontaneously diabetic rats.

2 Endothelium-dependent relaxation produced by acetylcholine or the calcium ionophore, A23817, in aortic rings precontracted with phenylephrine was significantly attenuated in diabetic vessels.

3 Relaxations produced by sodium nitroprusside or adenosine in diabetic preparations were comparable to those in control vessels.

4 The results show that diabetes leads to a specific impairment of endothelial-dependent relaxation.

Introduction

Diabetics are afflicted with a predisposition to various cardiovascular diseases such as hypertension, atherosclerosis and thrombosis (Garcia *et al.*, 1974; Christlieb *et al.*, 1976). It has been suggested that some manifestations of cardiovascular deterioration in diabetes are a consequence of altered sensitivity and/or responsiveness of vascular smooth muscle to neurotransmitters and hormones (Weidmann *et al.*, 1979). In this respect the reactivity of the aorta of diabetic rats to constricting agents has been extensively studied (Brody & Dixon, 1964; Sullivan & Sparks, 1979; Owen & Carrier, 1980; Pfaffman *et al.*, 1982). However, only a few studies deal with the response to relaxing substances (Palik *et al.*, 1981).

Since the original observations of Furchgott & Zawadzki (1980) it has been repeatedly demonstrated that the vascular endothelium has an important role in the effect of many vasodilator substances (Furchgott, 1983). Previous work on endothelium-dependent relaxation in diabetic rats is inconclusive. One study (Oyama *et al.*, 1986) reported a diminished endothelium-dependent relaxation while another suggested an enhanced endothelium-dependent relaxation (White & Carrier, 1987). More recently, Wakabayashi *et al.* (1987) observed that endothelium-dependent relaxation did not differ between control and diabetic vessels. All of the above studies were performed on streptozotocin-induced diabetic rats. In view of the inconsistent results obtained in the chemically-induced diabetic rat the present work examines endothelial-dependent relaxation in a strain of spontaneously diabetic rats.

Methods

The Bio-Breeding (BB) rat was selected for use in this study because it is a model of spontaneous diabetes that closely resembles type I diabetes in man. It shares with the human disease an abrupt onset of hyperglycaemia, glycosuria, ketonuria, and weight loss, with death usually occurring within days after its onset unless insulin therapy is instituted (Nakhooda *et al.*, 1976). The BB rats were obtained from a colony maintained at the Banting and Best Institute, University of Toronto. These BB rats were initially derived by crossing Long Evans rats (RTI^u haplotype) with seventh generation BB rats from the University of Massachusetts, Worcester. Subsequently, the colony was maintained by random matings between a diabetic male and a non-diabetic female. The overall incidence of overt diabetes up to an age of 120 days is consistently over 80% and the age of detection of diabetes is 65 to 85 days. Diabetic rats received daily subcutaneous injections of approximately 1.0 u insulin per 100 g body weight. Venous blood was obtained from the inferior vena cava the day of the experiment and the plasma

Table 1 Body weight, tissue weight, age, and plasma glucose levels for control and diabetic rats

Variable	*Controls* ($n = 12$)	*Diabetic* ($n = 13$)
Body weight (g)	393 ± 6	281 ± 27*
Aorta wet weight (mg)	3.5 ± 0.1	3.0 ± 0.2*
Age (days)	146 ± 4	139 ± 12
Plasma glucose ($mmol\,l^{-1}$)	8.86 ± 0.47	23.30 ± 1.99*

Values are means ± s.e. mean; n, number of animals.
* Statistically different from control ($P < 0.05$).

glucose level was measured by the glucose-oxidase method (Sigma). Animals used in this study were diabetic for approximately 9 weeks. Age-matched littermates which did not develop diabetes were used as controls.

After administration of ether anaesthesia a section of the thoracic aorta between the aortic arch and the diaphragm was removed, trimmed free of connective tissue and cut into transverse rings of 4 mm length. In some experiments, vessels were mechanically denuded by rubbing the intimal surface with fine forceps (DeMey & Vanhoutte, 1981). The aortic ring was mounted in a 20 ml organ bath containing a modified Krebs-Henseleit buffer containing ($mmol\,l^{-1}$): NaCl 117.56, $NaHCO_3$ 25.00, KCl 5.36, NaH_2PO_4 1.17, $CaCl_2$ 1.25, $MgSO_4$ 1.16, dextrose 11.10, glycerol 9.90 and sodium lactate 5.30. The incubation fluid was continuously aerated with a gas mixture of 95% O_2/5% CO_2; pH of this solution was 7.4, and temperature was maintained at 37°C. These tissues were attached to Grass FT 03C transducers connected to a Grass Model 7 oscillograph and isometric tension was recorded. Preliminary experiments were performed in which the resting tension was varied and contractile responses to phenylephrine (2.5 μM) were measured. The results indicated that a resting tension of 1 g was optimal for both diabetic and control tissues. Therefore, the arteries were equilibrated for 60–90 min under a resting tension of 1 g, during which time the bath was rinsed every 30 min with control buffer solution, and the tension was adjusted to 1 g.

When basal tension was stable, cumulative dose-response curves were performed using the contractile agonist, phenylephrine. Contractile responses were expressed as milligrams of force per milligram of tissue wet weight. For the relaxation studies the vessels were precontracted with 2.5 μM phenylephrine. This dose produced 80–85% of the maximal response. When the phenylephrine-induced contraction reached a plateau level, relaxant drugs were added in a cumulative fashion. Relaxation responses were expressed as the percentage of decreased tension of contractile force elicited by 2.5 μM phenylephrine. At the end of the experiment the tissues were blotted dry and weighed.

The following drugs were used in the present study: (−)-phenylephrine hydrochloride (Sigma), acetylcholine bromide (Sigma), sodium nitroprusside (BDH), adenosine (Sigma), calcium ionophore

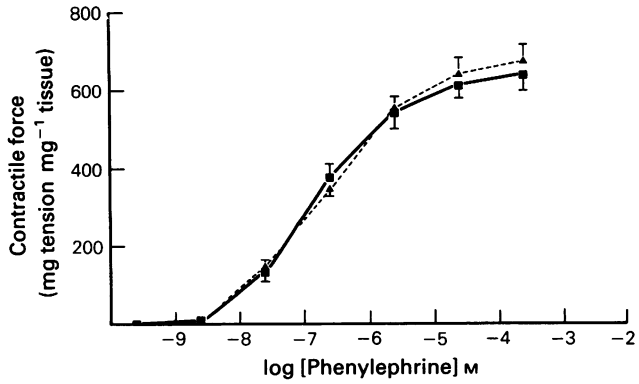

Figure 1 Cumulative dose-response curves to phenylephrine obtained from aortic rings of control (■, $n = 8$) or diabetic (▲, $n = 5$) rats. Means are shown with s.e. mean indicated by vertical lines.

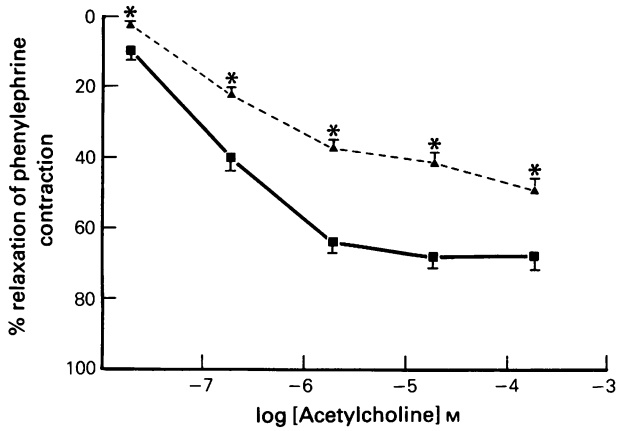

Figure 2 Cumulative dose-response curves showing the relaxant responses to acetylcholine in control (■) and diabetic (▲) aortae precontracted with phenylephrine (2.5 μM). Means ($n = 6$) are shown with s.e. mean indicated by vertical lines. * Statistically different from control ($P < 0.05$).

A23187 (Calbiochem), and protamine zinc insulin (Eli Lilly and Company). All concentrations are expressed as final molar concentrations of the base in the organ bath. In each protocol the number of preparations studied was also the number of rats used. Results are expressed as means ± s.e. mean. Significance was tested by unpaired Student's t tests and considered significant if $P < 0.05$.

Results

Approximately 9 weeks after the onset of diabetes, body weights and aortic tissue weights were significantly less in the diabetic rats compared to age-matched control animals (Table 1). In addition, the diabetic animals exhibited a marked hyperglycaemia.

The contraction of aorta in response to phenylephrine is depicted in Figure 1. There was no significant difference in the force of contraction induced by phenylephrine between the control and diabetic rings. When aortic rings were precontracted with phenylephrine (2.5 μM), acetylcholine induced a concentration-dependent relaxation of the precontracted aorta. However, the response was significantly attenuated in the diabetic aorta (Figure 2). Maximal relaxations to acetylcholine in control and diabetic rats were 68.1 ± 4.6% and 49.4 ± 2.3% of the contraction to phenylephrine, respectively ($n = 6$, $P < 0.05$). Furthermore, the relaxation produced by the calcium ionophore A23187 (0.5 μM) was also substantially reduced in the diabetic aorta (Figure 3). When control and diabetic aortae were mechanically denuded of endothelial cells, both the acetylcholine and the A23187-induced relaxation were abolished. In rubbed aortic rings in a relaxed state, similar small constrictor responses to acetylcholine (0.2 mM) were observed in both diabetic and control rings. No contractile effects were observed at lower doses of acetylcholine (data not shown).

In contrast to acetylcholine and A23187, however, dose-dependent relaxation produced by sodium nitroprusside or adenosine in diabetic aortae showed no shift and no depression of maximal relaxation capacity (Figures 4 and 5). Furthermore, removal of the endothelium did not alter the relaxation in either diabetic or control vessels (data not shown).

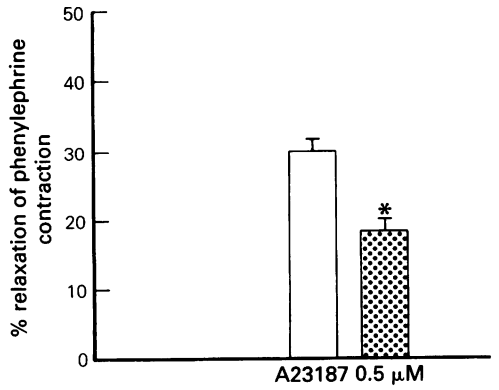

Figure 3 Relaxant effect of A23187 (0.5 μM) in control (open column) and diabetic (stippled column) aortae precontracted with phenylephrine (2.5 μM). Means ($n = 3$) are shown with s.e. mean indicated by vertical lines. * Statistically different from control ($P < 0.05$).

Discussion

The responses of blood vessels from diabetic animals to relaxing substances has been investigated in only

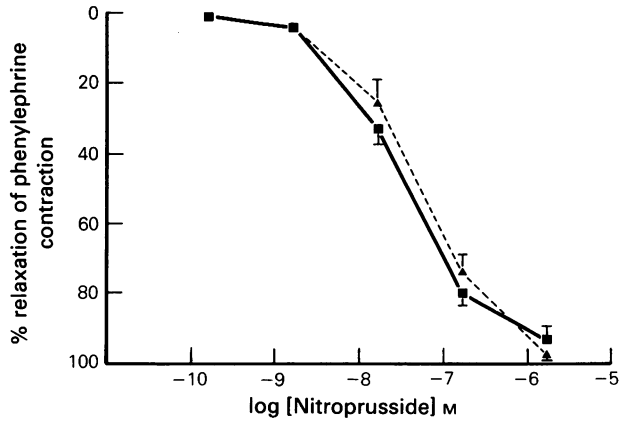

Figure 4 Cumulative dose-response curves showing the relaxant responses to sodium nitroprusside in control (■) and diabetic (▲) aortae precontracted with phenylephrine (2.5 μM). Means ($n = 7$) are shown with s.e. mean indicated by vertical lines.

a few studies (Palik *et al.*, 1981; Koltai *et al.*, 1984). Relaxing substances are known to fall into two classes. Some of them, such as nitroprusside and adenosine relax vascular smooth muscle in a direct endothelium-independent way, whereas others such as acetylcholine and A23187 mediate their effect via the endothelial cells (Furchgott, 1983). The present study showed that relaxation of the contracted aortic rings from diabetic rats was specifically depressed in response to the endothelium-dependent relaxing substances, acetylcholine and A23187, whereas the dose-response curves to the endothelium-independent substances nitroprusside and adenosine exhibited no change. It thus appears that there exists a characteristic depression of the relaxant responses to endothelium-dependent substances rather than a generalized decreased relaxing capacity for diabetic smooth muscle.

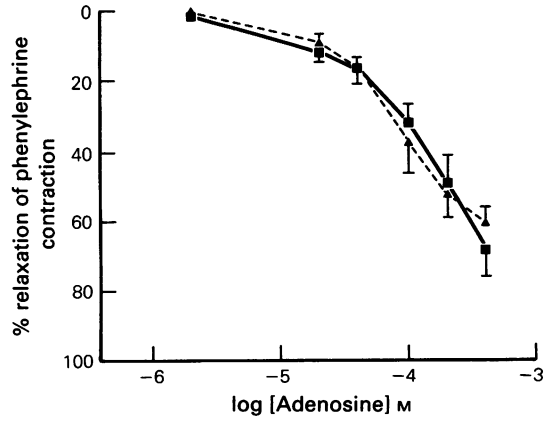

Figure 5 Cumulative dose-response curves showing the relaxant responses to adenosine in control (■, $n = 5$) and diabetic (▲, $n = 4$) aortae precontracted with phenylephrine (2.5 μM). Means are shown with s.e. mean indicated by vertical lines.

The discovery by Furchgott & Zawadzki (1980) that acetylcholine can relax vascular smooth muscle indirectly via the release of an endothelium-derived relaxant factor (EDRF) has expanded the awareness that vascular endothelial cells can release substances that can influence vascular tone. Once produced, EDRF diffuses from the endothelium to the smooth muscle, stimulating guanylate cyclase, leading to elevated levels of cyclic GMP which initiates the process of relaxation (Holzmann, 1982; Rapoport & Murad, 1983). Recent reports suggest that EDRF is nitric oxide (NO). Like EDRF, NO is a powerful vasodilator which acts directly on the smooth muscle (Khan & Furchgott, 1987). In addition, it has been shown that endothelial cells in culture release NO and that NO released from these cells is indistinguishable from EDRF in biological activity, stability and susceptibility to inhibiting and potentiating agents (Palmer *et al.*, 1987).

A possible explanation for the impairment of endothelial-dependent relaxation by acetylcholine in diabetic aorta may be a reduced production and release of EDRF. Thus, a diabetes-related abnormality in the synthesis of NO might result in the diminished relaxation observed in diabetic rats. Alternatively, disturbances in the transport of EDRF to smooth muscle cells and accelerated destruction of EDRF in the diabetic state may also play a role. Disturbances in both acetylcholine receptor density and transduction mechanism on diabetic endothelial cells might be a possible cause of the attenuated effects of acetylcholine on the diabetic aorta. However, this seems unlikely since the calcium ion-

ophore, A23187, which causes endothelium-dependent relaxation in a manner unrelated to any receptor mechanisms (Furchgott, 1983) also produced an attenuated response in the diabetic preparations. A final possible explanation for impaired endothelial-dependent relaxation in diabetes may include an alteration in the ability of diabetic smooth muscle to respond to EDRF.

Several studies indicate that blood vessels of diabetic animals are hypersensitive to pressor agents (Scarborough & Carrier, 1983; MacLeod & McNeill, 1985). Acetylcholine, apart from its endothelium-mediated relaxant effect, contracts vascular smooth muscle cells of isolated preparations in a direct way (Furchgott, 1955). Therefore, it could be that the decreased relaxation responses of aortic rings from diabetic rats to acetylcholine is due to an increased sensitivity of the smooth muscle cells to the direct contractile effects of acetylcholine. However, our results on relaxed endothelium-depleted preparations demonstrated that acetylcholine elicited a contractile response only at a high concentration (0.2 mM) and that this contractile effect on aortic rings from the diabetic animals was not potentiated.

In summary, this study provides evidence for an impaired relaxation of blood vessels from spontaneously diabetic rats in response to the endothelium-dependent relaxant agent, acetylcholine. Although the importance of the release of EDRF in affecting vascular resistance, especially in the case of acetylcholine, has yet to be clearly established it appears that the synthesis and release of EDRF may be altered in diabetes. Interestingly, recent reports have documented depressed endothelium-mediated vasodilator responses in various vascular disease states (Winquist *et al.*, 1984; Jayakoby *et al.*, 1985).

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