Diabetologia (1992) 35: 1011–1019

Diabetologia

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Originals

Impaired contraction and relaxation in aorta from streptozotocin-diabetic rats: role of polyol pathway

N. E. Cameron and M. A. Cotter

Department of Biomedical Sciences, University of Aberdeen, UK

Summary. The effects of 3 months streptozotocin-induced diabetes mellitus on contraction and relaxation of aorta were examined in vitro. A further diabetic group was treated with a novel sulphonylnitromethane-based aldose reductase inhibitor for 3 months following diabetes induction. Diabetes resulted in reduced maximal tension production, particularly for responses to phenylephrine ($p < 0.001$) and serotonin ($p < 0.001$). However, with aldose reductase inhibitor treatment, responses were in the non-diabetic range. The ratio of maximum contractions to noradrenaline and phenylephrine were 28% elevated by diabetes ($p < 0.01$), which may suggest increased α_2-adrenoreceptor-mediated responses. Endothelium-independent relaxation to glyceryl trinitrate was unaffected by diabetes or treatment. By contrast, there were 38% deficits in endothelium-dependent relaxation to acetylcholine ($p < 0.001$) and Ca^{2+} ionophore A23187 ($p < 0.001$) with diabetes which were prevented by aldose reductase inhibitor treatment ($p < 0.001$). A 121% shift in the concentration giving a 50% maximum effect for acetylcholine towards lower sensitivity with diabetes ($p < 0.001$) was also largely corrected by treatment ($p < 0.001$). A non-diabetic group treated with aldose reductase inhibitor showed a 30% decrease in the 50% effective concentration for acetylcholine ($p < 0.05$). A 15% deficit in maximum relaxation to the ATP-sensitive K^+ channel opener cromakalim for the diabetic group ($p < 0.001$) was prevented by aldose reductase inhibitor treatment ($p < 0.01$). We conclude that there are polyol pathway related abnormalities for contraction, some aspects of endothelium-independent relaxation, but particularly for endothelium-dependent relaxation in aorta from chronic streptozotocin-diabetic rats. If found in the appropriate circulatory beds, these could potentially contribute to the putative vascular basis of some of the complications of diabetes. Their amelioration could account for many of the beneficial effects of aldose reductase inhibitors.

Key words: Angiopathy, endothelium, endothelium-derived relaxing factor, nitric oxide, polyol pathway, aldose reductase, contraction, vascular smooth muscle, streptozotocin, diabetic rat.

In recent years a number of studies have suggested that the ability of vascular endothelium to relax smooth muscle [1] is impaired in human [2] and experimental diabetes. Animal models include streptozotocin (STZ) induced [3–9], or spontaneously diabetic rats [10–12] and alloxan-treated rabbits [13, 14]. There are, however, conflicting reports in the literature, with normal relaxation for chemically-induced diabetes in dogs, rabbits and rats [15–17], and deficits for spontaneously, but not chemically-induced, diabetic rats [12]. In addition, several explanations have been proposed ranging from a deficit in production of the endothelium-derived relaxing factor (EDRF) nitric oxide (NO) [3–6], to abnormal synthesis of eicosanoid vasoconstrictors [9, 13]. Furthermore, vascular reactivity may be enhanced in diabetic rats, perhaps because of increased α-adrenoreceptor-mediated responses [18–20]. Altered reactivity may be a separate effect rather than a consequence of abnormal endothelium function [21].

Vascular abnormalities probably contribute to the aetiology of many diabetic complications including neuropathy, nephropathy, retinopathy and myopathy [22, 23]. For example, there is an early reduction in nerve blood flow in STZ-diabetic rats which gives rise to conduction deficits that may be restored by vasodilator treatment [24–26]. There is also substantial evidence that nerve function [27–29] as well as aspects of nephropathy [22], retinopathy [30] and myopathy [31, 32] are preventable in diabetic rats by treatment with aldose reductase inhibitors (ARIs). There have been no reports that ARIs have haemodynamic effects in non-diabetic rats, which suggests that it is unlikely that they have a general vasodilator action. However, ARI treatment has been reported to ameliorate the reduction in nerve blood flow with diabetes

[33], prevent increases in vascular permeability [34], and partially correct a deficit in depressor responses to arachidonic acid [35]. Clinical trials with the ARI tolrestat also produced statistically significant reductions in systolic and diastolic blood pressure in diabetic patients [36]. Thus, ARIs may have vascular-related effects which may contribute to their beneficial actions on diabetic complications. This report investigates that possibility by examining the effects of preventive ARI treatment on contraction and relaxation of rat aorta following 3 months of STZ-diabetes.

Materials and methods

Mature male Sprague-Dawley rats, aged 19 weeks, from the Aberdeen University colony were used. All animals were housed under the same conditions and had food and water ad libitum. Two groups were made diabetic with a single i.p. injection of STZ (45 mg/kg) in citrate buffer (pH 4.5). One group was not treated for 3 months, which acted as a diabetic control group. The other group was treated from induction of diabetes for 3 months with a novel ARI, (4-amino-2,6-dimethylphenyl-sulphonyl) nitromethane, (ICI Pharmaceuticals, Macclesfield, Cheshire, UK) dissolved in the drinking water such that the final dose was approximately 21 $mg \cdot kg^{-1} \cdot day^{-1}$. This had no effect on the 250 ml/day drunk by each diabetic rat. Onset and age-matched non-diabetic control groups were also used. A further group of non-diabetic rats was given ARI treatment for 3 months, the concentration in the drinking water being adjusted to the same final daily dose as for the treated diabetic group. Blood glucose levels and glycosuria were monitored weekly (Visidex II and Diastix; Ames, Slough, Bucks., UK) for diabetic rats. Final plasma glucose values were measured on samples taken when the rats were killed.

In final experiments, thoracic aortas were removed under 2–5% halothane anaesthesia, cleaned of fat and connective tissue, cut into 4 mm rings, then cut longitudinally to form rectangles which were mounted using small tissue clips in 20 ml organ baths. They were bathed in modified Krebs-Ringer solution (144.0 Na^+, 5.0 K^+, 2.5 Ca^{2+}, 1.1 Mg^{2+}, 25.0 HCO^-, 1.1 PO_43-, 1.1 SO_42-, 11.0 glucose, in mmol/l) gassed with 95% O_2 5% CO_2 (pH 7.35) at 37°C. Tension was monitored using isometric transducers, the outputs being displayed on chart recorders. Resting tension was maintained at 1 g. The aortas were allowed to equilibrate for 45 min and were then tested for contraction with 1 µmol/l phenylephrine. They were then washed and left to equilibrate for a further 30 min. Bathing fluid was changed every 20–30 min except during cumulative dose-response experiments. Relaxation was assessed against a background of precontraction by phenylephrine (100–300 nmol/l) adjusted for individual tissue samples to give approximately 60% of maximal tension. At the end of the experiment, whilst still under tension, the length of tissue was measured with vernier callipers. Aortic rectangles were gently blotted to remove excess bathing medium, and weighed. Cross sectional areas were calculated from length and weight, assuming a tissue density of 1.05 [4], and developed tensions were expressed per cross sectional area.

Chemicals used came from the following sources: acetylcholine, Ca^{2+} ionophore A23187, serotonin, noradrenaline, phenylephrine, flurbiprofen and STZ were obtained from Sigma, (Poole, Dorset, UK). Glyceryl trinitrate, in 5 mg tablets, was obtained from Aberdeen Royal Infirmary Pharmacy. ARI, cromakalim and L-N^G-nitroarginine were gifts from ICI Pharmaceuticals.

Tissue sugars and polyols and assessment of ARI efficacy

Sciatic nerve and abdominal aorta polyol levels were estimated from samples taken immediately after dissection and frozen in liquid N_2. Trimethyl-silyl derivatives were prepared from aqueous deproteinized extracts and analysed by gas chromatography [37]. The sciatic nerve was used as a test tissue to ascertain the efficacy of polyol pathway blockade by ARI, as metabolites accumulate to high levels with diabetes. Results for the groups in this study are shown in Table 1. Compared to the control groups, sorbitol and fructose were elevated 16-fold in the non-treated diabetic group. With ARI treatment, for controls, sorbitol was 12% and fructose was 22% of the normal values. In the ARI-treated diabetic group sorbitol levels were 42% of those for non-diabetic controls and fructose was within the normal range. Nerve glucose was increased eight-fold with diabetes and this was unaffected by ARI treatment. Taken together, these results suggest a high level of polyol pathway blockade by the ARI. Aortic tissue is unlike nerve in that sorbitol does not accumulate to a great extent. This appears to be because cells are relatively permeable to this metabolite which is lost to the circulation, thus, when galactose is used as a substrate for the pathway, galactitol accumulates to similar levels to those found in sciatic nerve and these may be reduced by ARI in a dose-dependent fashion [22]. Similar observations have been made for cardiac and skeletal muscle [23, 31, 38]. Sorbitol levels of the aorta were 0.020 ± 0.005 nmol/mg wet weight in the non-diabetic controls ($n = 14$). This was increased to 0.068 ± 0.009 nmol/mg wet weight with the non-treated diabetic group ($p < 0.01$, $n = 16$). With ARI treatment sorbitol levels were 0.031 ± 0.010 nmol/mg wet weight, not significantly different from controls, but reduced compared to the non-treated diabetic group ($p < 0.05$, $n = 12$). In the ARI-treated control group, sorbitol levels were 0.010 ± 0.005 nmol/mg wet weight ($n = 5$). This suggests that ARI treatment was effective in blocking aorta polyol pathway activity.

Table 1. Sciatic nerve sugar and polyol levels

Group	n	Glucose	Sorbitol	Fructose
Control	15	2.11 ± 0.11	0.170 ± 0.010	0.505 ± 0.036
Control + ARI	5	1.96 ± 0.04	0.021 ± 0.002[a]	0.113 ± 0.008[a]
Diabetic	10	15.03 ± 2.16[a]	2.721 ± 0.220[a]	8.083 ± 0.655[a]
Diabetic + ARI	9	16.79 ± 1.02[a]	0.072 ± 0.003[b]	0.661 ± 0.041[b]

[a] $p < 0.001$ vs control group; [b] $p < 0.001$ diabetic + ARI vs diabetic group
Values are means ± SEM in nmol/mg tissue wet weight
ARI, Aldose reductase inhibitor

Statistical analysis

Results are expressed as group means ± SEM. Data were subjected to one-way analysis of variance, and any statistically significant differences were assigned to individual between group comparisons using Student's t-tests and the Bonferroni correction for multiple comparisons (Instat; GraphPad, San Diego, Calif., USA). To avoid unnecessary multiple comparisons, data from the ARI-treated control group were not compared with data from either diabetic group.

Results

Rats were mature at the start of the study, onset controls weighed 501 ± 12 g ($n = 17$) and age-matched controls reached 538 ± 13 g ($n = 12$). Diabetic animals lost weight, being 358 ± 15 g ($n = 22$) without and 395 ± 12 g ($n = 19$) with ARI treatment. The ARI-treated non-diabetic group weighed 582 ± 12 g ($n = 11$). Plasma glucose was elevated by diabetes to 33.4 ± 3.6 mmol/l, and was unaffected by ARI treatment (33.6 ± 3.8 mmol/l).

Contractile responses

There were no significant differences between onset and age-matched non-diabetic controls, and the data have been pooled for graphic presentation. Contractions of

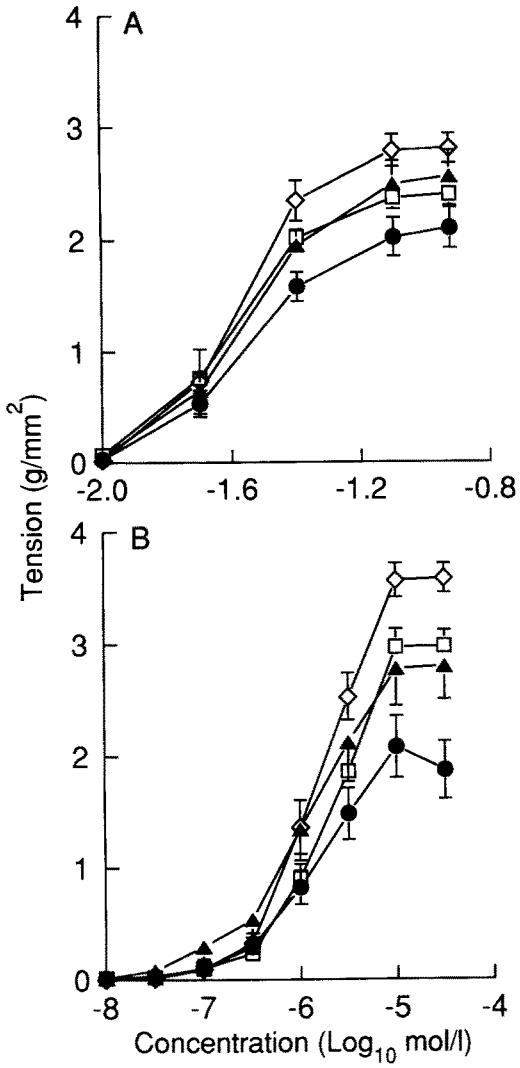

Fig. 1. A, tension development of aortas in response to K^+ for control (onset $n = 14$, age-matched $n = 10$, □), diabetic ($n = 16$, ●), aldose reductase inhibitor. (ARI)-treated diabetic ($n = 13$, ▲), and ARI-treated control ($n = 9$, ◊) groups. **B,** serotonin responses for control (onset $n = 13$, age-matched $n = 10$, □), diabetic ($n = 15$, ●), ARI-treated diabetic ($n = 15$, ▲) and ARI-treated controls ($n = 11$, ◊). Data are means ± SEM

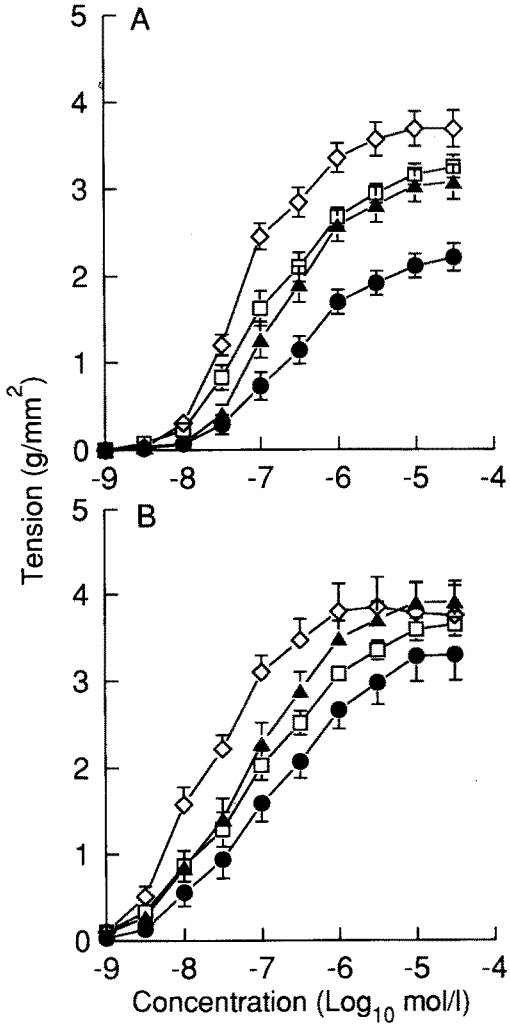

Fig. 2. A, B Tension development in response to phenylephrine (**A**) and noradrenaline (**B**) in aortas from control (onset $n = 13$, age-matched $n = 10$, □), diabetic ($n = 15$, ●), aldose reductase inhibitor (ARI)-treated diabetic rats ($n = 15$, ▲) and ARI-treated control rats ($n = 11$ for phenylephrine, $n = 6$ for noradrenaline, ◊). Data are means ± SEM

aorta from non-treated diabetic rats tended to be weaker than for controls. This was least apparent for K^+ contractures (Fig. 1 A) where the only significant difference ($p < 0.05$) was seen at 40 mmol/l K^+. Data points for the ARI-treated diabetic group were closest to those of controls, but there were no significant effects of treatment compared to the diabetic group. ARI-treated controls did not show any significant differences compared to non-treated controls. There were no significant differences in the effective concentrations for a 50 % maximal response (EC_{50}) which were 26.1 ± 1.4, 29.0 ± 2.2, 27.7 ± 2.2 and 27.8 ± 1.9 mmol/l for control, diabetic, ARI-diabetic and ARI-control groups respectively.

A diminished maximum contractile response to serotonin (Fig. 1 B) was noted with non-treated diabetes ($p < 0.001$). Contractile force was variable, however, with around 33 % of diabetic rats showing normal responses, whereas 67 % had responses at least 1 SD reduced from the normal range. This was not restricted to individual aortic rectangles, but was reproducible within a diabetic rat. Some specimens showed a tendency to relax at the highest concentration employed. Variability was also evident for the ARI-treated group, but the majority (74 %) of responses were in the normal range. There was a significant ($p < 0.05$) effect of ARI treatment only at the highest concentration (30 µmol/l). ARI-treated controls produced 19 % greater maximal tension than non-treated controls ($p < 0.05$). There were no significant differences in the EC_{50} between groups, values ranging from 1.98 ± 0.34 to 2.78 ± 0.80 µmol/l.

Responses to phenylephrine are shown in Figure 2 A. With diabetes, there was a 28 % reduction in maximum tension production ($p < 0.001$), and significant differences from control values between 30 nmol/l ($p < 0.05$) and 30 µmol/l ($p < 0.001$). ARI treatment largely prevented this, from 300 nmol/l ($p < 0.05$) to 30 µmol/l ($p < 0.01$). The ARI-treated diabetic group showed no significant differences from controls. ARI-treated controls did not

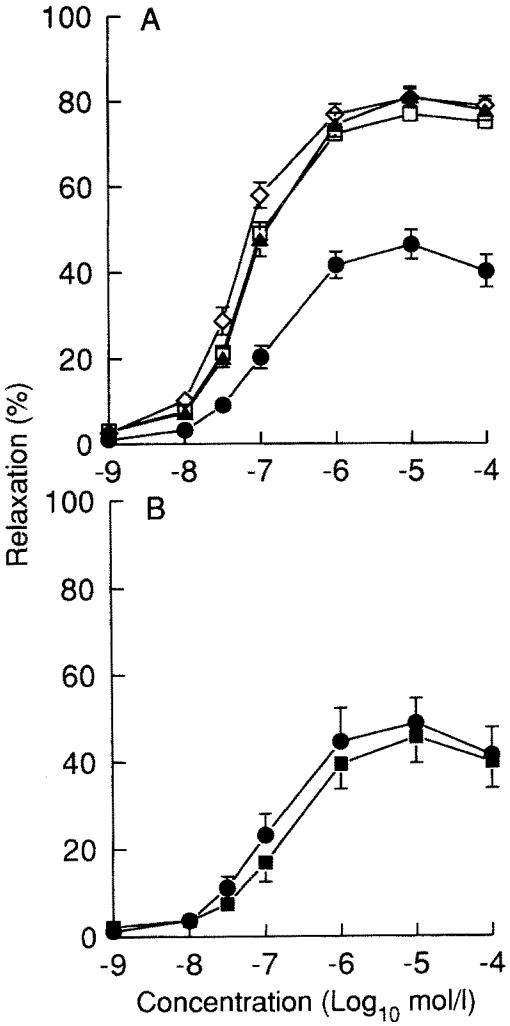

Fig. 3. A, B Relaxation in response to acetylcholine after pre-contraction with phenylephrine. **A,** controls (onset $n = 14$, age-matched $n = 10$; □), diabetic controls ($n = 22$; ●), aldose reductase inhibitor (ARI)-treated diabetic group ($n = 19$; ▲), ARI-treated non-diabetic group ($n = 11$; ◊). **B,** diabetic controls without ($n = 10$; ●) or after 30 min pre-treatment with 5 μmol/l flurbiprofen ($n = 10$, ■). Data are means ± SEM

differ significantly from non-treated controls for maximal contraction, however, responses for intermediate concentrations of phenylephrine (100 nmol/l to 10 μmol/l) were significantly greater ($p < 0.05$). There were no significant effects on EC_{50} which ranged from 69 ± 11 nmol/l for ARI-treated controls to 313 ± 63 nmol/l for non-treated diabetes. In the same tissue samples, responses to noradrenaline were also measured (Fig. 2B). No clear deficit with diabetes was apparent, and there were no significant differences from controls. The ARI-treated diabetic group also did not differ from non-diabetic controls, however, there was an increase in tension production compared with diabetic controls between 0.3 and 3 μmol/l ($p < 0.05$). There was no significant effect of ARI-treatment on control maximum contraction, however, at intermediate concentrations (30 nmol/l to 1 μmol/l) of noradrenaline responses in the ARI-treated group were enhanced ($p < 0.05$). There were no significant differences in EC_{50}.

The ratio of maximum tension production stimulated by noradrenaline compared to phenylephrine was 1.431 ± 0.092 for the diabetic group, whereas for non-diabetic controls it was 1.116 ± 0.029 ($p < 0.01$). For ARI-treated diabetic rats, the ratio was 1.276 ± 0.057, not significantly different from either group. For ARI-treated controls, the ratio was the same as for non-treated controls (1.115 ± 0.030).

Relaxation

Maximum endothelium-dependent relaxation was 38% impaired ($p < 0.001$) in diabetic aorta when stimulated with acetylcholine (Fig. 3A). This was prevented by ARI treatment ($p < 0.001$), the data points showing no significant departures from normal. Maximal relaxation was not affected in ARI-treated controls, although relaxations were significantly greater ($p < 0.05$) between 30 nmol/l and 100 nmol/l compared to non-treated controls. There was a 121% shift in EC_{50} towards lower sensitivity with diabetes (75.2 ± 5.1 vs 166.1 ± 15.3 nmol/l, $p < 0.001$), which was largely (75%) prevented by ARI treatment (98.0 ± 7.4 nmol/l, $p < 0.001$). For ARI-treated controls there was a small (30%) shift in EC_{50} towards greater sensitivity (52.6 ± 5.3 nmol/l, $p < 0.05$) compared to non-treated controls.

Maximum relaxation to acetylcholine in a subset of the diabetic group was not affected by pre-incubation for 30 min with a cyclooxygenase inhibitor (5 μmol/l flurbiprofen) (Fig. 3B). However, there was a 61% increase in EC_{50} ($p = 0.0031$, paired t-test) from 142.2 ± 23.1 to 229.3 ± 28.6 nmol/l. Acetylcholine relaxations were not found after the endothelium had been removed (not shown), and in intact aorta from both control and diabetic rats, maximal responses to 10 μmol/l acetylcholine were completely reversed by 10 μmol/l L-N^G-nitro arginine [39].

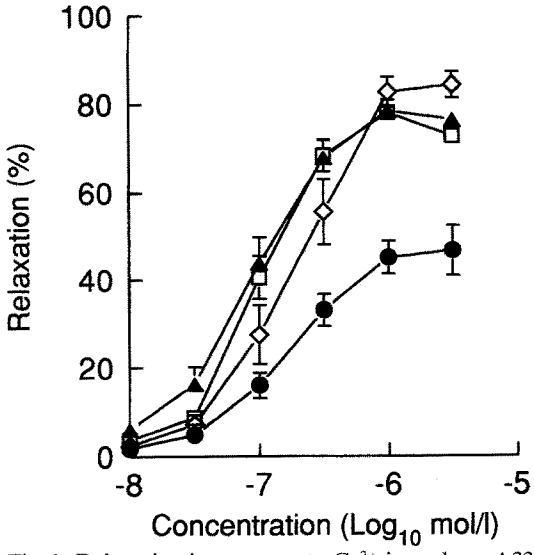

Fig. 4. Relaxation in response to Ca^{2+} ionophore A23187 after pre contraction with phenylephrine. Controls (onset $n = 10$, age-matched $n = 8$; □), diabetic controls ($n = 14$, ●), aldose reductase inhibitor (ARI)-treated diabetic rats ($n = 12$, ▲) and ARI-treated controls ($n = 6$, ◊). Data are means ± SEM

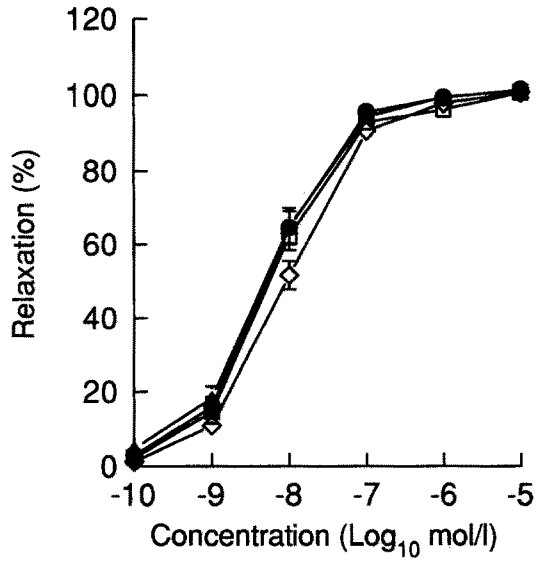

Fig. 5. Relaxation in response to glyceryl trinitrate after pre contraction with phenylephrine. Controls (onset $n = 10$, age-matched $n = 9$; □), diabetic controls ($n = 10$, ●), aldose reductase inhibitor (ARI)-treated diabetic rats ($n = 10$, ▲) and ARI-treated controls ($n = 6$; ◊). Data are means ± SEM

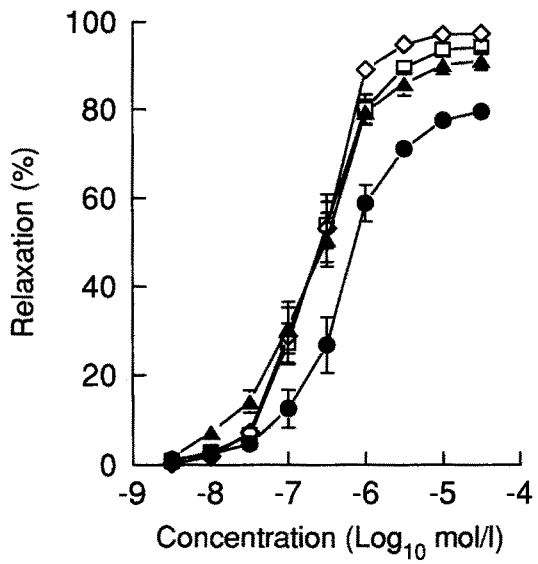

Fig. 6. Relaxation in response to cromakalim after pre-contraction with phenylephrine. Controls (onset $n = 10$, age-matched $n = 10$; □), diabetic controls ($n = 11$, ●), aldose reductase inhibitor (ARI)-treated diabetic rats ($n = 14$, ▲), and ARI-treated controls ($n = 10$, ◊). Data are means ± SEM

Concentration-response curves for relaxation with Ca^{2+} ionophore A23187 are shown in Figure 4. Ionophore-mediated relaxations were similar in magnitude to those seen for acetylcholine, and provide evidence for an ARI-preventable diabetic deficit of around 38% ($p < 0.001$ for the control and ARI-treated diabetic vs the non-treated diabetic group). There were no significant differences between ARI-treated and non-treated controls. EC_{50} values (range 129 ± 27 to 291 ± 89 nmol/l) were not significantly affected by diabetes or treatment.

There were no significant between-group differences in relaxation mediated by glyceryl trinitrate (Fig. 5) for either EC_{50} (range 5.7 ± 1.1 to 7.1 ± 1.5 nmol/l) or maximum relaxation (range 100.4 ± 0.7% to 102.3 ± 1.0%).

Responses to the ATP-sensitive K^+ channel opener cromakalim are shown in Figure 6. This resulted in a maximum 91–97% relaxation of control, ARI-treated diabetic and ARI-treated control aortas at higher concentrations. However, in non-treated diabetic aortas the maximum relaxation was approximately 80%, significantly reduced compared to the control or ARI-treated diabetic groups ($p < 0.001$ and $p < 0.01$, respectively). ARI treatment had no effect on cromakalim-induced relaxation in non-diabetic rats. There were no significant differences in EC_{50} which were in the range of 279 ± 54 (ARI-treated controls) to 519 ± 87 nmol/l (non-treated diabetic group).

Discussion

The data show that ARI treatment can partially prevent contraction and almost completely prevent relaxation deficits in aorta from diabetic rats, without affecting hyperglycaemia or body weight loss. This has not previously been demonstrated. It is unlikely that diabetic changes resulted from a non-specific cytotoxic effect of STZ, because both contraction and relaxation deficits may be reversed by insulin treatment [7], and have been demonstrated in genetically susceptible diabetic BB-Wistar rats [10–12].

Contractions tended to be weaker with diabetes, particularly for phenylephrine and serotonin, without significant changes in sensitivity as reflected by EC_{50} values. This partially agrees with some other reports in the literature [7, 16, 40, 41], although sensitivity may vary with diabetes duration [42]. For noradrenaline, there was no significant deficit with diabetes. There have been reports of decreased responses [7, 16, 43], no effect [17], and increased sensitivity and/or contractile force [4, 18, 44]. While disagreements may depend on diabetes duration and severity, elevation of the noradrenaline/phenylephrine tension ratio supports the notion of increased α_2-mediated responses [18, 19]. One possible explanation is that diabetes caused changes in adrenoreceptor expression. ARI-treatment may have had a partial preventive effect, although statistically the results were equivocal. In another system, the ARI ponalrestat prevented an increase in myocardial β-receptor-mediated sensitivity in diabetic rats [45]. An alternative explanation is that α-adrenoreceptor-mediated contraction is partially offset by stimulation of EDRF release. α_2 stimulation normally evokes fairly equal contraction and relaxation so its contribution is minimized. However, with endothelial damage or dysfunction, a net contraction is produced which is additive to the α_1 component when both are stimulated by noradrenaline. This view is supported by the data of Wakabayashi et al. [19] who compared responses to an α_2 agonist in aorta with and without endothelium from diabetic and non-diabetic rats.

The reasons for reduced tension production with diabetes are unclear, particularly as results were agonist-dependent. Cardiac and skeletal muscle show impaired Ca^{2+}

handling by sarcolemma and sarcoplasmic reticulum [46–48], and the functional consequences of this are prevented by ARI treatment [31, 32]. Contractile responses of aorta from diabetic rats show increased susceptibility to low extracellular Ca^{2+}, indicating greater reliance on this source [44], and release from intracellular stores may also be impaired [49]. Since contractile strength was normal in the ARI-treated diabetic group, it is plausible that a polyol pathway related deficit in Ca^{2+} homeostasis could affect tension production. Interpretation is complicated, however, by the evidence that aorta from ARI-treated non-diabetic rats tended to produce greater tensions than non-treated controls, even when the data were normalized for tissue cross sectional area. An explanation is not obvious, but it could reflect a polyol pathway influence on Ca^{2+} handling even in normoglycaemia.

The reduction in endothelium-dependent relaxation is in agreement with several studies on aorta from STZ-diabetic rats [3–7], although others have failed to find a deficit [12, 16, 17, 21]. The cause for the discrepancy is unclear, however, age and diabetes severity may be factors. The largest effect was evident when diabetes was induced in mature male rats [4]; a similar model was used in this investigation. Genetically susceptible BB-Wistar rats develop insulin-dependent diabetes when they are relatively mature, and all studies found impaired endothelium-dependent responses [10–12].

The cause of the endothelial deficit is unknown. There have been reports of patchy aortic endothelium damage [4, 12], and marked morphological changes have been noted in cerebral arterioles, including 20 % necrosis of endothelial cells and mitochondrial swelling, after 4 weeks of STZ-diabetes [50]. However, functional defects of aorta, without obvious damage, have also been described [3, 6]. Thus, metabolic abnormalities may be the prominent early cause of dysfunction. In rabbit aorta and rat cerebral arterioles, hyperglycaemia induces abnormal endothelial production of PGH_2/TxA_2 in response to acetylcholine the result of which causes contraction to oppose relaxation. Ca^{2+} ionophore A23187 does not evoke this response, and it may be prevented by an appropriate receptor blocker or cyclooxygenase inhibitor [9, 13]. The abnormality may even be induced in non-diabetic rabbit aorta in vitro by exposure to elevated glucose levels for several hours [51]. This mechanism is not responsible for dysfunction in diabetic rat aorta, as flurbiprofen did not improve relaxation, and there was a deficit for Ca^{2+} ionophore A23187, which agrees with a previous report [11]. Responses to glyceryl trinitrate were unaffected by diabetes, in agreement with other in vitro studies employing nitrodilators [4, 5, 11], with one exception [12]. Thus, the data suggest that the abnormality relates to the production/release of NO, rather than to deficits in acetylcholine receptor/transduction or smooth muscle guanylate cyclase systems.

ARI treatment almost completely prevented endothelium-dependent abnormalities. There are several mechanisms which potentially explain this effect. Endothelium possesses polyol pathway activity, and, when cultured under high glucose conditions, cells accumulate sorbitol [52]. Aldose reductase requires NADPH as a cofactor [22]. Thus, when polyol pathway flux is enhanced by hyperglycaemia, competition with other NADPH-requiring enzymes will occur. This results, for example, in diminished levels of reduced glutathione (GSH) in several tissues [22]. NADPH is a cofactor for NO synthase, which controls production of NO from L-arginine [53] and would, therefore, be subjected to competition from the polyol pathway leading to deficits in endothelium-dependent relaxation. An indication that this might occur even for modest levels of glucose availability is given by the shift in acetylcholine EC_{50} values for non-diabetic controls with and without ARI treatment. Tissue ARI levels remain elevated long after treatment has ceased and plasma levels decline, presumably due to binding to aldose reductase [54]. Thus, it is likely that the polyol pathway remained inhibited in aortas from ARI-treated controls during the experiments. The 11 mmol/l glucose in the Krebs-Ringer solution should have provided a background polyol pathway flux to influence NO production, and inhibition would be expected to enhance sensitivity to agonists.

Another polyol-pathway-related change that could influence relaxation concerns a reduction in ATP synthesis, because agonist-stimulated EDRF release requires metabolic energy. Thus, inhibitors of both oxidative and glycolytic metabolism reduce NO synthesis in aorta from non-diabetic rabbits and rats, and cultured porcine endothelial cells [55–57]. When polyol pathway flux is high, glucose is diverted through the pentose phosphate shunt [22]. This process requires ATP, and for each molecule of glucose that takes this route, there is a net deficit of three molecules of ATP [58]. This deficit in energy metabolism with diabetes may be sufficient to impair NO production.

Decreased levels of GSH as a result of polyol pathway activity, and other diabetic changes including reduced superoxide dismutase which depends in part on the GSH abnormality [59], compromise tissue protection against oxidative stress and superoxide radicals. Cultured human endothelial cells in a high glucose concentration medium showed diminished glutathione-redox-cycle-related handling of peroxide, which was ameliorated by ARI treatment [60]. In addition, auto-oxidative glycosylation of proteins would be expected to generate oxygen free radicals [61]. Endothelium from diabetic rats is abnormally sensitive to superoxide damage [4]. Superoxide also breaks down NO [62], and in vitro addition of superoxide dismutase partly restores endothelium-dependent relaxation in aorta from diabetic rats [6]. Thus, cumulative effects of diabetes on endothelial integrity and function are likely, in part related to polyol pathway flux.

A longer term mechanism concerns formation of advanced glycosylation end products (AGEs). Chemically, these can act to quench NO. After 2 months of diabetes, blood pressure responses to acetylcholine and glyceryl trinitrate are impaired in rats [63]. Similar functional deficits have been noted for diabetic patients [64]. In diabetic rats this may be prevented by aminoguanidine treatment, which inhibits AGE formation [63]. Unfortunately, interpretation may be somewhat more complex, as recent work suggests that aminoguanidine also has ARI activity [65]. However, the direct (fructose) and indirect (fructose 6-

phosphate and pentoses) products of polyol pathway activity are considerably more reactive than glucose in forming AGEs. Thus, ARIs could slow the development of AGE-related endothelial dysfunction, such as intracellular NO quenching and potentially deleterious effects for enzymes such as NO synthase.

The finding of an impairment in relaxation to the ATP-sensitive K^+ channel opener cromakalim with diabetes is in agreement with a previous study [66], but is difficult to account for. It could reflect a relative paucity of ATP-sensitive K^+ channels, or an alteration in the mechanisms relating hyperpolarization to closure of voltage-dependent Ca^{2+} channels, or indeed a defect in the Ca^{2+} channels themselves. It has been suggested that diabetes enhances the activity of Ca^{2+} channels in vascular smooth muscle [67]. Whether this deficit would have functional consequences is unclear. ATP-sensitive K^+ channels could have a protective role when intracellular ATP levels are low [68], for example due to ischaemia, and subsequent vasodilation when channels open could be beneficial for tissue perfusion. This would suggest that under some circumstances diabetic tissues might be more susceptible to ischaemic damage, and that ARI treatment might provide protection via this mechanism.

If the effects seen for aorta generalize to resistance vessels in some vascular beds, then they could contribute to the complications of diabetes. In vivo measurements on conscious rats show that after 4 weeks of diabetes the reduction in hindlimb vascular resistance following bradykinin and enothelin-1 stimulation of endothelium is attenuated, as is the increase in vascular resistance due to infusion of the NO synthesis blocker N^G-nitro-L-arginine methyl ester [69, 70]. For sciatic nerve, endoneurial blood flow is 40% reduced within one week of diabetes induction [24]. Myocardial perfusion is also impaired by diabetes [71]. Vasodilator treatment prevents nerve conduction deficits and some of the contractile changes in hindlimb skeletal muscles [24–26]. These are also prevented by ARI treatment [31, 32]. This suggests that an important mechanism of ARI action in these tissues could be to correct polyol pathway related vascular dysfunction, particularly of the endothelium. A plausible conclusion is that this could explain the beneficial actions of ARIs in ameliorating diabetic complications such as neuropathy, at least in experimental models. Recent work has emphasised that many of the metabolic events of diabetes cause vascular abnormalities [24, 34, 63], thus, the current dogmatic distinction between "metabolic" and "vascular" aetiologies of diabetic complications is becoming increasingly untenable.

Acknowledgements. We thank Dr. F. Carey of Imperial Chemical Industries for valuable discussion and support, and for supplying the ARI, and D. Mirrlees and Ms J. Stafford for polyol analysis.

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Received: 29 April 1992
and in revised form: 30 June 1992

Dr. N. E. Cameron,
Department of Biomedical Sciences,
University of Aberdeen,
Marischal College,
Aberdeen AB9 1AS, UK