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Changes in Isoprenaline-Induced Endothelium-Dependent and -Independent Relaxations of Aorta in Long-Term STZ-Diabetic Rats: Reversal Effect of Dietary Vitamin E

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ABSTRACT. 1. The present study concerns *in vitro* isoprenaline (ISO)-induced relaxation of aortic rings of long-term streptozotocin (STZ)-diabetic and nondiabetic rats, both with and without dietary vitamin E supplementation.

2. Incubation with propranolol, N^G-nitro-L-arginine methyl ester and methylene blue, as well as absence of endothelium, all negatively affect the ISO-induced relaxations.

3. Thiobarbituric acid reactivity levels used as an index of lipid peroxidation are elevated in the aorta by diabetes. Four months of STZ-diabetes results in a significant increase in the ISO-induced relaxations together with endothelial dysfunction in the rat aorta. Diabetes also causes the loss of vascular integrity.

4. Dietary vitamin E supplementation during the last 2 months of diabetes allows normalization of the levels of lipid peroxides. This vitamin also completely reverses the increased sensitivity (pD_2 value) of the aorta to ISO, whereas the maximum ISO-induced relaxations are partially restored after the treatment in diabetic rats.

5. The results suggest that ISO-induced relaxation in the aorta partially depends on the intact endothelium and that the endothelium-dependent relaxant effect of ISO is mediated by endothelium-derived relaxing factor. Results also indicate that abnormal vascular reactivity and structure of the diabetic rat aorta may be related to the increased lipid peroxidation. In conclusion, vitamin E can protect the arterial wall from oxidative stress–induced injury associated with chronic STZ-diabetes and allows normalization of the response to ISO and the structure of the aorta in diabetic rats. GEN PHARMAC 29;4:561–567, 1997. **© 1997 Elsevier Science Inc.**

KEY WORDS. Thoracic aorta, vitamin E, STZ-diabetes, endothelium, nitric oxide, isoprenaline, lipid peroxidation, electron microscopy

INTRODUCTION

Relaxation responses of the blood vessels to isoprenaline (ISO) are mediated by β-adrenoceptors on the smooth muscle and are conventionally endothelium independent (Heeson and De Mey, 1990; Kukovetz *et al.*, 1981). In this regard, Moncada *et al.* (1991) found that ISO-induced relaxation in the rat aorta is neither endothelium dependent nor affected by inhibitors of NO synthase. Contrary to the expectation, some reports suggested that endothelium may play a facilitatory role in the relaxation response to β-adrenoceptor agonists. These include the demonstration that removal of the endothelium from the rat aorta (Kamata *et al.*, 1989) and canine coronary arteries (Rubanyi and Vanhoutte, 1985) attenuates ISO-induced relaxation. Similarly, it was demonstrated in the rat aorta that ISO-induced relaxations are inhibited by methylene blue (MB) or by hemoglobin (Grace *et al.*, 1988), agents known to affect the action of NO (Martin *et al.*, 1985). Other studies showed that ISO-induced relaxations are attenuated by NO synthase inhibitors in the rat aorta (Gray and Marshall, 1991) and in rat mesenteric resistance arteries (Graves and Poston, 1993). Interestingly, Gray and Marshall (1992) showed that ISO-induced relaxations totally depend on the presence of intact endothelium in the rat thoracic aorta. Owing to the discrepancies among the previous reports, the first aim of the present study was to investigate whether intact endothelium does in fact mediate relaxations induced by ISO in the rat thoracic aorta.

Diabetes mellitus is known to produce alterations in the responsiveness of vascular smooth muscle to various vasoactive agents (Karasu and Altan, 1993). This is an important reason for the development of cardiovascular disease associated with diabetes (Karasu and Altan, 1993; MacLeod and McNeill, 1985). Accordingly, most investigators have demonstrated that endothelial dysfunction accompanies the increased responsiveness of vessels to vasoconstrictor agents in experimental diabetic rats (Abiru *et al.*, 1990; Karasu and Altan , 1994). To our knowledge, there is one study, reported by Kamata *et al.* (1989), investigating the effects of chemically induced diabetes on the response of the aorta to β-adrenoceptor agonists. The second aim of the present study, therefore, was to determine the effects of long-term STZ-diabetes on ISO-induced relaxations.

On the other hand, the exaggerated free radical activity and in-

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creased lipid peroxidation with a reduction in plasma vitamin E content have been well documented in diabetes (Chisolm *et al.*, 1992; Kunisaki *et al.*, 1990). These mechanisms are central to the development of the diabetes-induced micro- and macroangiophatic diseases (Giugliano *et al.*, 1995). We have consistently found that vitamin E treatment is beneficial in the protection of vascular (Karasu *et al.*, 1997) and neuronal (Karasu *et al.*, 1995) functions against streptozotocin (STZ)-diabetes–induced injury. However, the effectiveness of vitamin E supplementation on ISO-induced relaxations in diabetic rats has not been questioned. For this reason, a third aim of the present study was to investigate whether the long-term diabetes-caused changes in ISO-induced relaxations in the rat aorta can be reversed by the dietary supplementation of vitamin E as a strong inhibitor of lipid peroxidation (Esterbauer *et al.*, 1991).

MATERIALS AND METHODS

Experimental organization

The experiments were carried out on male albino rats with a starting weight of 200–250 g and an age range of 8–10 weeks. In the experiments, the animals were randomly assigned to four experimental groups. Age-matched nondiabetic rats were employed as onset control group. Nondiabetic control rats were fed a standard rat diet or a diet supplemented with vitamin E (DL-α-tocopheryl acetate 0.5% w/w) for 2 months. Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg, freshly dissolved in sterile 0.9% agueous NaCl.). Two days later, diabetes was verified by estimating hyperglycemia in tail vein blood (Ames Glucometer, Miles Laboratories Inc., Elkhart, IN, USA). Rats having blood glucose levels of 15 nmol/l or higher were considered to be diabetic. One group of diabetic rats was untreated for 4 months to act as a diabetic control. Other diabetic rats were untreated for 2 months and then treated for another 2 months with vitamin E (0.5% w/w by diet).

Preparation of tissue

Rats were weighed, anesthetized with diethyl ether and injected with heparin sodium (200 U IV). The thoracic aorta was quickly excised and placed in Krebs-Ringer bicarbonate buffer solution with the following composition (in mM): NaCl, 118.5; KCl, 4.74; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO4, 1.18; $NaHCO_3$, 24.9 and glucose 10.0. The aorta was cleaned of fat and connective tissue and cut into rings approximately 3–4 mm long. One ring of each pair was left intact; in the other ring, the endothelium was mechanically removed as described previously (Karasu and Altan, 1993). The rings with or without endothelium were mounted horizontally with the use of a pair of stainless hooks in tissue baths for tension measurement. Tissue baths were filled with 20 ml of Krebs solution continuously bubbled with a mixture of 5% CO_2 95% O_2, pH 7.4, at 37°C. Tension was measured by an isometric force transducer (Ugo Basile, No. 7004, Varese, Italy) connected to a microdynamometer (Ugo Basile, Unirecord).

Relaxation studies

The rings were equilibrated for 90 min, under a resting tension of 2 g. During the equilibration period, the solution in the tissue bath was replaced every 30 min. At the end of this period, successful removal of the endothelium was verified by the lack of relaxation response to acetylcholine (ACh; 10^{-5} M) in preconstricted rings. To measure ISO- or sodium nitroprusside (SNP)-induced relaxations, the rings were preconstricted to their EC_{75} value with noradrenaline (NA) (Rubino *et al.*, 1995) to obtained a stable plateau, and then cumulative concentration-response curves to ISO (10^{-9}–10^{-4} M) and SNP (10^{-10}–10^{-6} M) were obtained in rings with or without endothelium. Some rings with endothelium obtained from untreated control rats were incubated with MB (10^{-6} M for 30 min) or N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M for 10 min) before preconstriction. To confirm the involvement of β-adrenoceptors in isoprenaline-induced relaxation, the effect of propranolol, a nonselective β-antagonist, was investigated. At least 30-min intervals were allowed between consecutive concentration-response curves, during which time the Krebs' solution was changed at least three times.

Histology

Immediately after dissection, aortic rings from each animal were cut into small pieces, fixed at 4°C for 2 hr in 2.5% buffered glutaraldehyde and postfixed for 1.5 hr with 1% osmium tetraoxide. Tissues were dehydrated in ethyl alcohol followed by propylene oxide and embedded in araldite. The specimens were then counterstained with uranyl acetate and lead citrate and examined in a Carl-Zeiss electron microscope.

Biochemical measurements

Blood samples were taken from the tail vein for plasma glucose determination (GOD-Perid method; Boehringer, Mannheim) just before final experiments.

Measurement of malondialdehyde (MDA) by thiobarbituric acid (TBA) reactivity is the most widely used method for assessing lipid peroxidation. Lipid peroxidation was determined by using 200 μl of aortic homogenate as previously described (Jain and Levine, 1995). Incubations were carried out in triplicate and terminated by addition of 0.5 ml of tricloroacetic acid and 0.25 ml 1% TBA. After incubation in a boiling water bath for 15 min, the samples were centrifuged for 15 min and absorbencies of the supernatant read at 548 nm. The results were expressed in terms of MDA equivalents. Freshly prepared MDA tetramethyl acetal solution was used as a standard.

Drugs

The following pharmacological agents were used: STZ, ACh chloride, NA bitartarat, ISO, MB, L-NAME hydrochloride, propranolol hydrochloride and SNP (Sigma, St. Louis, MO, USA) and DL-α-tocopheryl acetate (a gift from Roche Company, Istanbul, Turkey).

Data analysis

The relaxation response to agonists was expressed as a percentage of the precontraction induced by NA. The additional effect of the endothelium on ISO-induced relaxation was evaluated as a difference between the corresponding values of maximal relaxation in rings with or without endothelium, which was called the *endothelial response* (ER). We calculated the *percentage of endothelial response* (PER) (Karasu *et al.*, 1997) with the following formula:

$$\text{PER} = \frac{100 \times \text{ER}}{\text{Maximum relaxation of ring with endothelium (g tension/mg tissue)}}.$$

Agonist pD_2 values (apparent agonist affinity constant: $-\log ED_{50}$) were calculated from each agonist dose-response curve by linear regression analysis of the linear portion of the curve and taken as a measure of the sensitivity of the tissue to each agonist. All values are expressed as means±SEM; n=number of animals. Statistical differences between means were assessed by either unpaired or paired t-test as appropriate. For multiple comparisons, analysis of variance followed by Neuman-Keul's test was used.

TABLE 1. General characteristics of rats

	Control (n = 10)	Control + vit. E (n = 10)	Diabetic (n = 9)	Diabetic + vit. E (n = 10)
Final body weight (g)	476 ± 12.0[b]	487 ± 18.8[b]	242 ± 9.2[a]	268 ± 11.5[a]
Plasma glucose (mmol/l)	7.4 ± 0.9[b]	6.6 ± 1.2[b]	38.8 ± 4.5[a]	41.6 ± 8.0[a]

[a]$P < 0.01$; statistically different from age-matched treated or untreated controls.
[b]$P < 0.01$; statistically different from age-matched untreated diabetics.

RESULTS

Body weights and plasma glucose concentrations of rats, and TBARS levels of aortae

The general characteristics of rats are shown in Table 1. Diabetic rats gained no weight during the experiments, whereas nondiabetic control rats almost doubled in weight; vitamin E treatment had no effect on the body weight of diabetic rats. Four-month diabetes caused hyperglycemia. Vitamin E treatment of diabetic rats had no significant effects on the increased plasma glucose.

Thiobarbituric acid reactive substances (TBARS) levels, which were used as an index of lipid peroxidation, were elevated by diabetes approximately threefold in the aorta compared with controls, but reversal–vitamin E treatment significantly decreased the raised aortic lipid peroxidation (Fig. 1).

Relaxation response to ISO and the effects of MB, L-NAME and propranolol

ISO caused relaxation in aortic rings with or without endothelium in a concentration-dependent manner when aortic rings were preconstricted with NA (Fig. 2). Mechanical removal of the endothelium significantly reduced the maximal relaxations produced by ISO in all experimental groups with the exception of the vitamin E–treated diabetic group. Similarly, incubation with MB (10^{-6} M) or L-NAME (10^{-4} M) significantly decreased the relaxant effects of ISO in aortae with endothelium from untreated control rats (see Fig. 2). The inhibitor effects of the removal of the endothelium or pretreatment either with MB or with L-NAME on the maximum relaxations produced by ISO in untreated-control aortic rings were similar (the maximum relaxation response to ISO was 76.8±4.3%, untreated control, versus 27.3±5.8%, L-NAME, n=5, P<0.001, paired t test; 76.8±4.3%, untreated control versus 31.5±3.8%, MB, n=7, P<0.001, paired t test), indicating that the endothelium-dependent relaxant effect of ISO was being mediated by NO. The relaxation response to ISO of rings preconstricted with NA was completely inhibited by propranolol (10^{-6} M) (see Fig. 2).

PER was 51.6±3.8% and 26.5±1.7% in untreated-control and untreated-diabetic rings, respectively (p<0.001, unpaired t test). The maximum relaxation responses and pD_2 values to ISO are shown in Table 2. The maximum relaxation and pD_2 value in response to ISO were significantly increased in untreated diabetic rats compared with controls. The concentration-response curve for ISO was shifted to the left for aortic rings from untreated diabetic rats. The treatment of rats with vitamin E did not alter the response to ISO in the control group. Vitamin E treatment of diabetes completely reversed the relaxation response to ISO only at lower doses of the agonist (10^{-9}–10^{-6} M), whereas the effect of diabetes on the maximum relaxation response to ISO was partially restored by this vitamin. There was no significant difference in the maximum relaxation response to ISO of vitamin E–treated diabetic aortae with or without endothelium compared with untreated-control or untreated-diabetic aortae with endothelium. The increased pD_2 value for ISO-induced relaxation in untreated-diabetic aortae was significantly reversed by vitamin E treatment.

SNP induced 100% relaxation, and the PD_2 values for SNP-induced relaxations in rings with or without endothelium were similar in all experimental groups (7.42±0.09, untreated control with endothelium versus 7.21±0.11, without endothelium, n=7, nonsignificant, unpaired t-test).

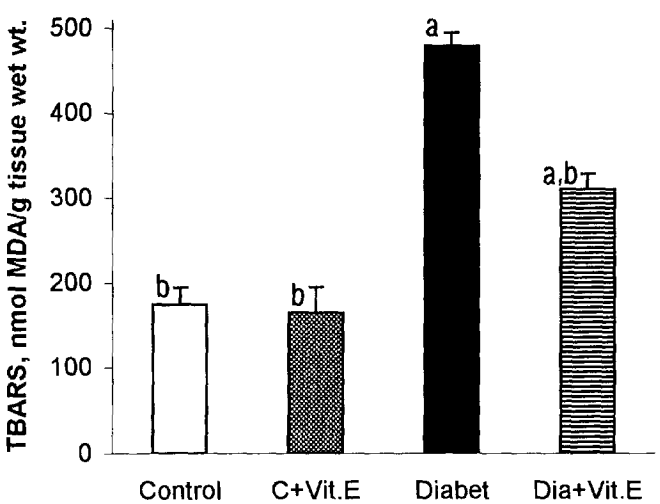

FIGURE 1. Lipid peroxidation levels as TBARS in aorta obtained from all experimental groups. Values are means±SEM. [a]P<0.01, statistically different from untreated control rats. [b]P<0.01, statistically different from untreated diabetic rats.

Histology

In aortic rings from control animals, the endothelium formed a continuous layer and no intimal lesions were noted (Fig. 3). Endothelial cells were smooth and uniform, and adhering white blood cells were not observed. The aortic media from control rats showed a regular disposition of the smooth muscle cells between the elastic lamina within a homogenous interstitial matrix. In aortic rings from untreated diabetic rats, the intimal surface appeared irregular and intimal lesions and local cytoplasmic edema in endothelial cells were observed. Thickened intima and lipid-laden foam cells were present in the diabetic aorta. An increase in myocyte size and thickened elastic laminae with degenerative interstitial matrix also were observed. STZ-diabetic animals treated with vitamin E also showed several intimal lesions, but the tunica media appeared slightly smoother with fewer defects.

DISCUSSION

The present study demonstrates that ISO causes relaxations in the rat aorta that partially depend on the presence of intact endothelium. These findings confirm the results of previous studies that ISO-induced relaxations are inhibited by mechanical removal of the endothelium (Grace *et al.*, 1988; Kamata *et al.*, 1989) or by pre-

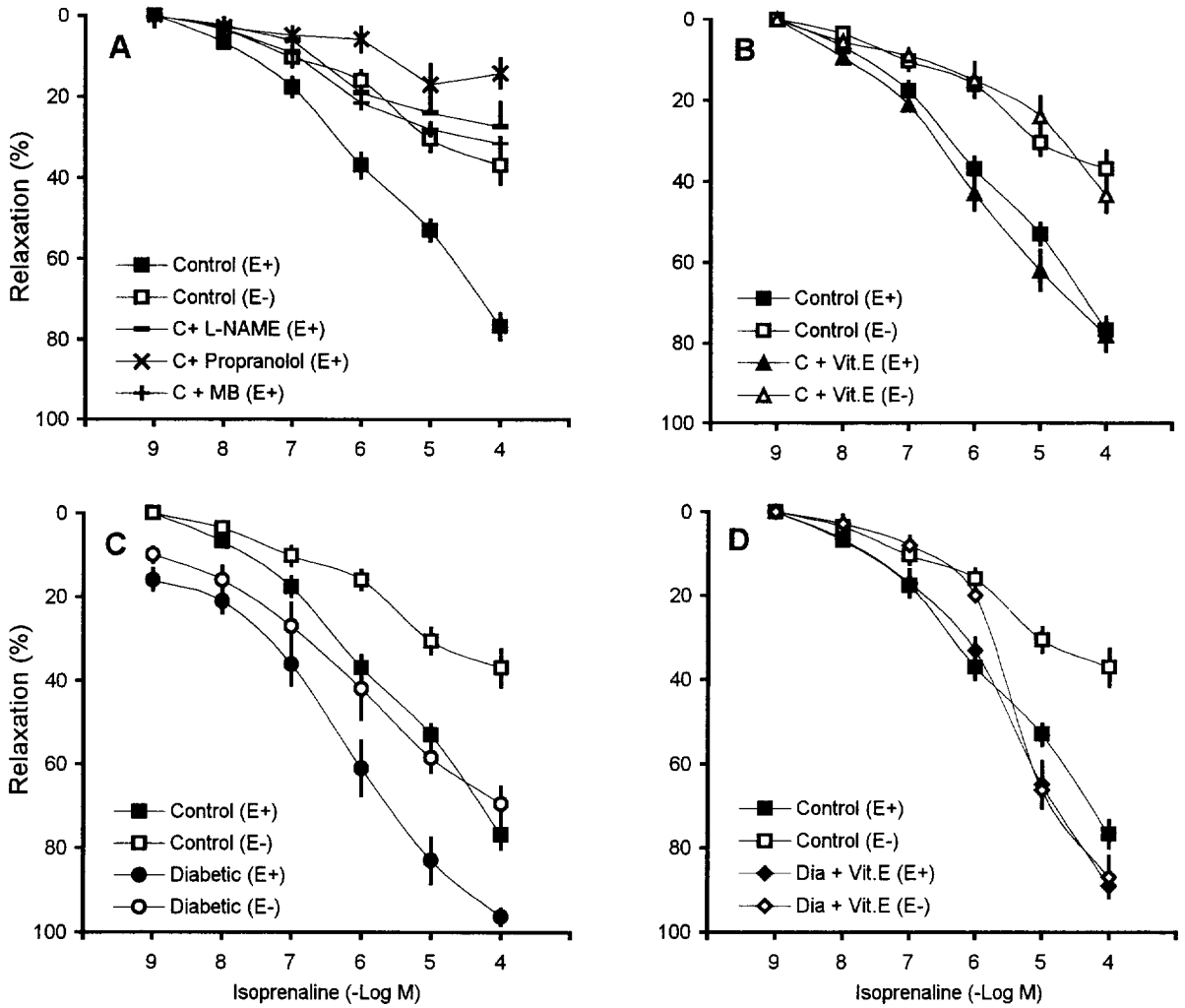

FIGURE 2. Cumulative concentration-response curves of ISO in preconstricted thoracic aorta with (E+) or without (E−) endothelium obtained from untreated control (control), vitamin E–treated control (C+Vit. E), untreated diabetic (diabetic), vitamin E–treated diabetic (Dia+Vit. E) rats. C+MB, ISO-induced relaxations in untreated control rat aorta after incubation with MB. C+L-NAME, ISO-induced relaxations in untreated-control rat aorta after incubation with L-NAME. Values are means±SEM.

treatment with either MB (Grace *et al.*, 1988) or L-NAME (Gray and Marshall, 1991) in the rat aorta. The magnitudes of the inhibitory effects of L-NAME, MB and absence of endothelium on ISO-induced relaxations are similar in preconstricted aortae from untreated control rats. Because previous studies showed that MB can inhibit soluble guanylate cyclase (Martin *et al.*, 1985) and L-NAME is a specific inhibitor of endothelium-derived relaxing factor (EDRF)/NO synthetase (Gardiner *et al.*, 1991), the preceding finding in our study provides the evidence that the mediating effect of endothelium on ISO-induced relaxations occurs through EDRF/NO. It is well known that the vascular endothelium is capable of controlling vessel tone by releasing many vasoactive substances, among them EDRF/NO, which stimulates muscle soluble guanylate cyclase and thereby increases levels of cyclic GMP (Furchgott and Vanhoutte,

TABLE 2. Maximum responses and pD_2 values for ISO-induced relaxations of aortic rings from rats

	Maximum relaxation (%)		pD_2	
	E+	E−	E+	E−
Control, $n = 6$	76.8 ± 4.33[b]	37.1 ± 2.73[a,b]	5.91 ± 0.09[b]	6.08 ± 0.08[b]
Control + Vit. E, $n = 6$	78.0 ± 4.16[b]	43.7 ± 8.15[a,b]	6.19 ± 0.02[b]	5.72 ± 0.25[b]
Diabet, $n = 7$	96.0 ± 1.81[a]	69.5 ± 1.78[b]	6.75 ± 0.24[a]	6.63 ± 0.18[a]
Diabet + Vit. E, $n = 8$	89.0 ± 2.35[a]	86.6 ± 5.28[b]	5.94 ± 0.08[b]	5.69 ± 0.09[b]

n = number of rats; E+, with endothelium; E−, without endothelium.
[a] $P < 0.01$; statistically different from untreated control rings with endothelium.
[a] $P < 0.01$; statistically different from untreated diabetic rings with endothelium.

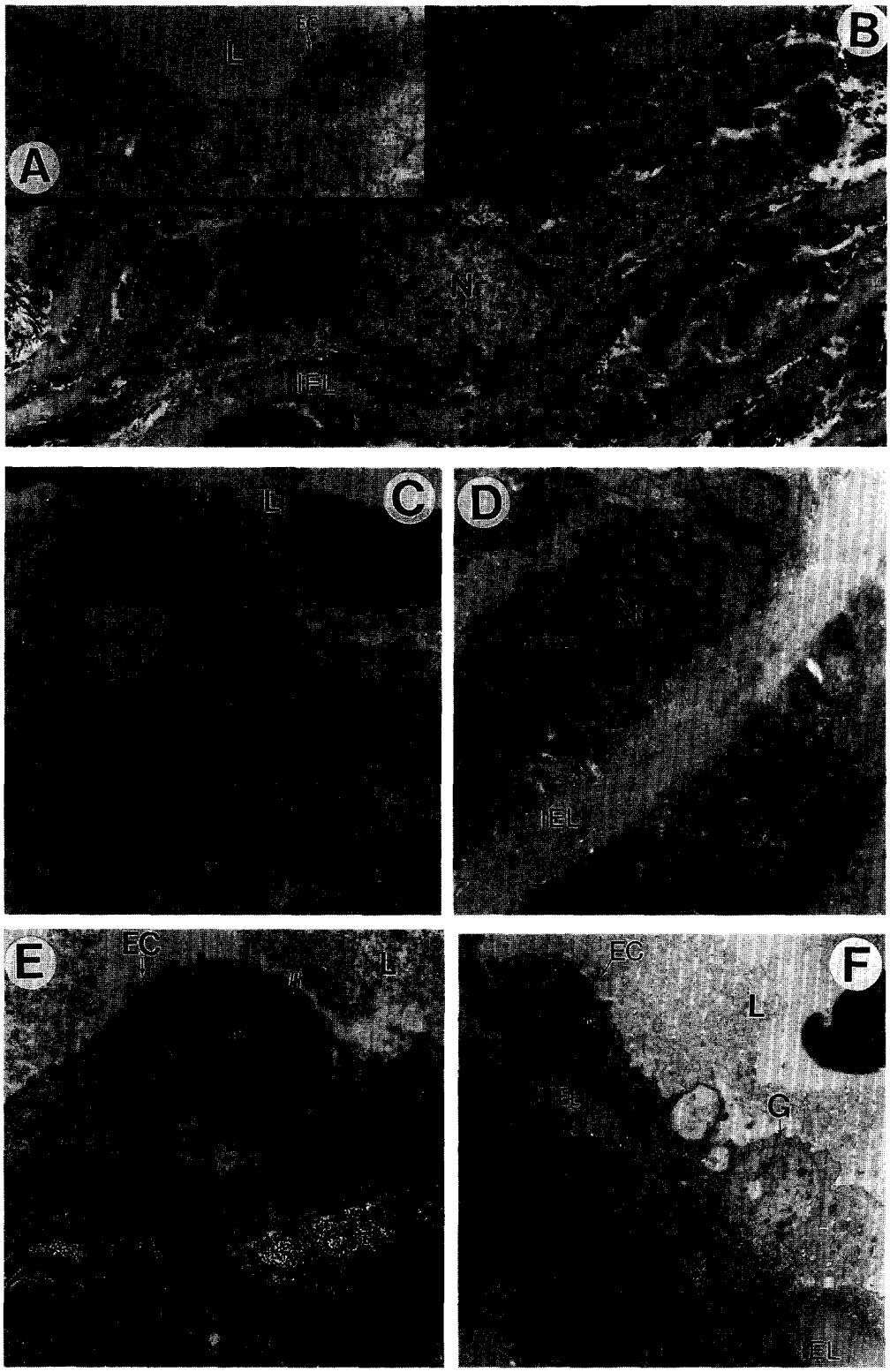

FIGURE 3. Electron micrographs of longitudinal sections of rat thoracic aortae. Representative examples of (A) an endothelial cell (EC) and (B) tunica media of an aorta taken from a nondiabetic control rat. Tunica media showed a regular position of smooth muscle cells (smc) between the internal elastic laminae (IEL) within a homogenous interstitial matrix; Co, collagen fibers. In untreated diabetic rats, endothelial cell of aorta (C) showed several cytoplasmic blebs protruding toward the lumen (two arrows). Cytoplasmic membrane has lost its integrity. In tunica media of untreated diabetic aorta (D), intracytoplasmic organization of collagen fibers has disappeared. The structure of smc appeared abnormal; irregular and thickened IEL can be seen. An endothelial cell of vitamin E–treated diabetic aorta (E) showed no large defect. Although cytoplasmic blebs (two arrows) existed, cytoplasmic membrane was regular and had a normal appearance. A portion of endothelial cell of vitamin E–treated diabetic aorta (F) presented a different pattern of cellular damage with a reduction in the cytoplasmic blebs. IEL has lost its integrity (*), and was infiltrated by a granulocyte (G). Tunica media was irregular, and there was a marked decrease in the thickness of IEL. N, nucleus. Uranyl acetate and lead citrate staining. Original magnifications for A, D, F × 3600 and for B, C, E × 9000.

1989). In the present study, the mediating effect of endothelium on ISO-induced relaxation is significantly attenuated by long-term STZ-diabetes. The lower PER in aortae from untreated diabetic rats determined in the present study clearly suggests that the endothelial dysfunction present may be related to be reduced EDRF/NO activity (Cohen, 1993).

A further interesting observation to emerge from this study is the effect of chronic STZ-diabetes on ISO-induced relaxations. STZ-diabetes causes marked elevations in both the sensitivity and the maximal response to ISO. These findings are inconsistent with the results of Kamata *et al.* (1989), who showed that β-adrenoceptor–mediated relaxation responses of aortic strips were significantly decreased in 8–10 week STZ-diabetic rats. The discrepancy between our results and those of Kamata *et al.* (1989), is not known but may be partly due to a difference in the preparation of tissues. The mechanism(s) by which chronic STZ-diabetes leads to an increase in ISO-induced relaxation is not clear. The relaxant response to SNP does not differ significantly in aortic rings from diabetic and control rats. Therefore, the increased responsiveness to ISO is not due to altered activity of guanylate cyclase in vascular smooth muscle. Interestingly, STZ-diabetes increases ISO-induced vascular relaxation both in the presence and in the absence of endothelium, and this event occurs in spite of the diabetes-induced endothelial dysfunction. ISO-induced relaxations are abolished by propranolol, confirming the involvement of β-adrenoceptors. Thus, it is possible to suggest that the increased relaxant response to ISO may be linked to the alterations in the postreceptor events and to the density or affinity or both of β-adrenoceptors on smooth muscle but not on endothelium.

Free radicals are generated in the cytosol during oxidative reactions, and their increased levels have been well documented in STZ-diabetic rats (Morel and Chisolm, 1989) and in humans with diabetes (Nishigaki *et al.*, 1981). These extremely reactive substances react with lipids within the cell membrane, leading to lipid peroxidation (Chisolm *et al.*, 1992). It has been proposed that free radical–induced oxidative damage is central to the development of micro- and macroangiopathic disease observed in diabetes (Baynes, 1992; Giugliano *et al.*, 1995). In this study, lipid peroxidation was assessed by measuring the tissue content of MDA, one of the end products of lipid peroxidation, and we determined the increased levels of MDA in untreated diabetic rats. Thus, the decreased contribution of the endothelium on ISO-induced relaxation in the untreated diabetic rat aorta is likely to result from an increased degradation of EDRF/NO by oxidative reactions rather than from defects in the other systems. Experimental evidence indicates that free radical–induced lipid peroxidation can induce endothelial cell injury and dysfunction (Henning and Chow, 1988; Tasfamariam, 1994). Furthermore, the exaggerated levels of reactive oxygen species in the vessel wall may also be a reason for the increased responsiveness of the diabetic aorta to ISO. It is possible that, as a consequence of free radical–induced oxidative reactions, changes in membrane fluidity alter membrane-associated processes such as carrier-mediated transport, passive diffusion and ligand-receptor interactions (Freeman *et al.*, 1986). There is an opinion that lipid peroxidation alters the fluidity of the lipid environment crucial to the integrity of receptors (Ebersole and Molinoff, 1991). On the other hand, Langenstroer and Pieper (1992) suggested that the chronic STZ-diabetic rat aorta releases more superoxide anion radical than does the control rat aorta, resulting in a greater inhibitory effect on the action of spontaneously released EDRF/NO. These authors have determined an elevation in catalase in the chronic STZ-diabetic rat aorta with no difference in the superoxide dismutase or glutathione peroxidase activity. They have also suggested that the increase in catalase activity reveals increased exposure of the diabetic aorta to hydrogen peroxide (H_2O_2), leading to increased relaxation of the diabetic rat aorta. Therefore, it may be speculated in the present study that long-term STZ-diabetes promotes H_2O_2 production by dismutating superoxide anion and that the increased production of H_2O_2 may be an appropriate explanation for greater ISO-induced vascular smooth muscle relaxation by activation of guanylate cyclase. We cannot totally believe that this mechanism is adequate for our model, because it is not clear at this time how ISO can stimulate the production of H_2O_2. Further studies should differentiate these possibilities.

The abnormal vascular reactivity observed in diabetic rats may be due partially to pathological changes in the vessel wall. Indeed, the results of the electron microscopy study showing typical abnormalities in the structure of aortic wall strongly support this hypothesis.

Furthermore, we examined the reversal effect of vitamin E on vascular abnormalities observed in STZ-diabetic animals. The treatment normalizes the increased sensitivity of the aorta to ISO in diabetic animals. The treatment also completely reverses the increased ISO-induced relaxation in the presence and absence of endothelium that is observed only at lower doses of the agonist (10^{-9}–10^{-6} M). However, the maximum relaxation of the aorta with or without endothelium still remained increased after treatment with vitamin E, which is significantly different from those of control or untreated diabetic rats. One likely explanation for vitamin E's effect may be associated with its antioxidant properties. Present results support this possibility because vitamin E can attenuate the lipid peroxidation in aortae of long-term diabetic rats. It is well known that vitamin E is the major antioxidant in low-density lipoproteins (LDL) and is among the first antioxidants to be consumed during LDL oxidation (Esterbauer *et al.*, 1991; Morel and Chisolm, 1989). Otherwise, the beneficial effect of vitamin E may be linked to its actions on the membrane, such as membrane fluidity (Urano *et al.*, 1988). On the other hand, vitamin E treatment of long-term diabetes does not appear to improve the endothelial dysfunction completely, because ISO-induced relaxation was not modified by the mechanical removal of the endothelium, at least at higher doses of the agonist. Thus, results suggest that prolonged therapy with vitamin E is required to completely reverse abnormal reactivity and structure of the diabetic aorta.

In conclusion, ISO-induced relaxation in the aorta partially depends on the existence of intact endothelium. Four-month STZ-diabetes induces an increase in ISO-induced relaxation together with endothelial dysfunction in the rat aorta. Dietary vitamin E during the last 2 months of STZ treatment partially restores the response to ISO and the structure of the aorta, and it significantly decreases the lipid peroxidation levels.

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