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Effects of calcium dobesilate on the synthesis of endothelium-dependent relaxing factors in rabbit isolated aorta

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1 Some cardiovascular disturbances which occur in diabetics are a consequence of alterations in vascular contractility as well as in endothelium-dependent relaxation.

2 Calcium dobesilate (DOBE) is a drug used in diabetic retinopathy and its mechanism of action is not yet understood.

3 The aim of this study was to investigate the effects of DOBE on synthesis and release of endothelium-dependent relaxing factor (EDRF) and endothelium-dependent hyperpolarizing factor (EDHF) in rabbit isolated aorta.

4 Endothelium-dependent relaxation induced by acetylcholine (ACh) (10^{-8}–10^{-5} M) increased in the presence of DOBE 10^{-5} M only when vascular endothelium was kept intact.

5 N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-8}–10^{-4} M progressively decreased the enhancing effect of DOBE on endothelium-dependent relaxation whereas it was progressively increased by L-Arg.

6 DOBE 10^{-5} M increased in a non-significant manner endothelium-dependent relaxation induced by ACh when the arteries were incubated with both L-NAME 10^{-4} M and indomethacin 10^{-6} M.

7 DOBE (10^{-6} M and 10^{-5} M) was able to scavenge superoxide anion radicals generated by the hypoxanthine/xanthine oxidase reaction.

8 These results provide evidence that DOBE is able to affect the vascular disorders associated with diabetes mellitus since it enhances the synthesis of endothelium-dependent relaxing factors.

Keywords: Calcium dobesilate; endothelium-dependent relaxation; aorta; EDRF; EDHF

Introduction

According to the W.H.O. 1% of diabetics die of diabetic comas, but as many as 74% die of vascular complications. It has been recognized that long-standing hyperglycaemia induces microvascular changes in the retina through a number of pathological mechanisms including capillary hyperpermeability, thickening of the capillary basement membrane and blood hyperviscosity (Little, 1983; MacMillan, 1985; 1989). In addition, it has been suggested that some of the cardiovascular disturbances which are known to occur in patients with diabetes mellitus (DM) are a consequence of alterations in the reactivity of blood vessels to neurotransmitters and circulating hormones (Weidman *et al*., 1979). Thus, MacLeod & McNeill (1985) have demonstrated that contractile responses to noradrenaline of the aorta and mesenteric arteries from rats with streptozotocin-induced diabetes, are enhanced possibly due to a decrease in the spontaneous release of endothelium derived relaxing factor (EDRF), resulting from damage to the endothelial cells (Harris & MacLeod, 1988). On the other hand, there are discrepancies in the data obtained on endothelium-dependent relaxation in arteries from diabetic animals. Wakabayashi *et al*. (1987) have shown that endothelium-dependent relaxation of the aorta in response to acetylcholine (ACh) is unaffected by the diabetic state. In contrast, Oyama *et al*. (1986) have found that endothelium-dependent relaxation of diabetic aortae is attenuated in relation to control. Further McVeigh *et al*. (1992) demonstrated an abnormal relaxation to ACh in the forearm of subjects with non-insulin dependent (type II) diabetes mellitus. A reduction in basal and stimulated nitric oxide (NO) synthesis in patients with insulin-dependent diabetes associated with microalbuminuria has also been demonstrated (Elliot *et al*., 1992).

Previous studies have shown that endothelium-dependent relaxation of diabetic vasculature is more sensitive to free radical-induced injury (Pieper & Gross, 1988). The abnormal endothelium-dependent relaxation in aorta from the diabetic rabbit was restored to normal by superoxide dismutase (SOD), suggesting a role for superoxide anions in the abnormality of endothelial cells caused by diabetes mellitus (Tesfamarian & Cohen, 1992).

Calcium dobesilate (DOBE) (calcium 2,5-dihydrosybenzene sulphonate), one of the most active members of a series of cyclohexadienolic bisulphate derivatives, is an orally administered angioprotective agent. It has been shown to lower blood, plasma and aqueous humor hyperviscosity (Barras & Gralt, 1980; Vojnikovic, 1984; Barras & Michel, 1986; Salama *et al*., 1987) reduce microvascular hyperpermeability (Beyer *et al*., 1980), inhibit platelet aggregation (Glovizki *et al*., 1985; Salama *et al*., 1985; Michal & Gotti, 1988) and has been successfully used in the treatment of chronic venous insufficiency (Hachen & Lorenz, 1982) and diabetic retinopathy (Salama *et al*., 1985; Leite *et al*., 1990). The aim of this study was to investigate the effects of DOBE on relaxing and contracting responses in rabbit isolated aorta and to determine whether some endothelium-derived factors are implicated in these actions.

Methods

General procedure

Male New Zealand white rabbits weighing 2.5–3.0 kg were obtained from Biocentre S.A. (Barcelona, Spain). The animals were anaesthetized with ethyl ether and killed by exsanguination from the common carotid artery.

Isolated aortic preparations

The thoracic aorta was rapidly removed and placed in Godfraind solution of the following composition (mM): NaCl 121,

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KCl 5.8, $NaHCO_3$ 14.9, $MgCl_2$ 1.22, glucose 11 and $CaCl_2$ 1.25. Adherent fat and surrounding tissue were cleaned off and the arteries were cut into rings approximately 2–3 mm wide. The rings were then suspended between two stainless steel hooks in organ baths containing 10 ml of Godfraind solution. The solution was kept at 36 ± 0.5°C and gassed continuously with a 95% O_2-5% CO_2 gas mixture. The aortic rings were mounted under 2 g tension. Each preparation was allowed to equilibrate for 90–120 min. Contractile responses were measured isometrically by means of force-displacement transducers (Grass FT 03) and were recorded on a Grass polygraph as previously described (Tejerina *et al*., 1988).

The arteries were divided into two groups, the endothelium was removed in one group of arteries (arteries −E), endothelium-denuded arteries were prepared mechanically by inserting a stainless-steel rod into the rings and rubbing the rings gently with our fingers, following the procedure described previously (Furchgott *et al*., 1984), whereas in the other group the endothelium was kept intact (arteries +E). After equilibration the following experiments were carried out.

(1) In order to investigate whether DOBE was able to enhance endothelium-dependent relaxation in aortic arteries an initial contraction to noradrenaline (NA) 10^{-6} M was obtained. When the plateau was reached, the following cumulative relaxation curves were made by adding aliquots of the relaxing agonist.

(1.1) Concentration-response curves to acetylcholine (ACh) (10^{-8}–10^{-5} M) were obtained. The curves were made in both groups of arteries (+E and −E). After wash-out, the rings were incubated with DOBE 10^{-5} M or 10^{-4} M for 45 min and the latter procedure was repeated. To elucidate whether the synthesis and release of either EDRF or/and EDHF (endothelial derived hyperpolarizing factor) could be implicated in the mechanism of action of DOBE, the arteries +E were contracted again with NA 10^{-6} M. When the plateau was reached a concentration-response curve to ACh 10^{-8}–10^{-5} M was constructed. This procedure was carried out before and after pre-incubation with N^G-nitro-L-arginine methyl ester (L-NAME) (10^{-8}–10^{-4} M) or L-arginine (L-Arg) (10^{-8}–10^{-4} M), in both experiments DOBE 10^{-5} M was present (we chose that concentration because it showed a maximal effect on endothelium-dependent relaxation). Moreover, we studied whether the effect of L-NAME was specific for nitric oxide synthase by trying to reverse the effect of L-NAME 10^{-6} M on endothelium-dependent relaxation with L-Arg 10^{-4} M plus DOBE 10^{-5} M. In addition, arteries +E were first contracted with NA 10^{-6} M and relaxed with ACh (10^{-8}–10^{-5} M), and then after wash out, were incubated in the presence of L-NAME 10^{-4} M plus indomethacin 10^{-6} M (to block prostacyclin synthesis) for 45 min, with or without DOBE 10^{-5} M.

(1.2) In another group of experiments, we investigated the next step in the EDRF pathway; soluble guanylyl cyclase stimulation by NO. Thus, we studied the effect of DOBE on both the inhibition and potentiation of the enzyme by use of an antagonist and a guanosine 3′:5′-cyclic monophosphate (cyclic GMP) analogue. Arteries +E were exposed to a submaximal concentration of NA (10^{-6} M) and ACh added (10^{-8}–10^{-5} M) to the bath when the plateau was reached. After wash out, the rings were incubated with DOBE 10^{-5} M, 1H-(1,2,4) oxadiazolo(4,3-a)quinoxaline-1-one (ODQ) (10^{-6} M) or 8Br-cyclic GMP (10^{-8}–10^{-4} M) plus DOBE 10^{-5} M for 45 min and the latter group of experiments was repeated.

(2) We also studied whether DOBE could scavenge superoxide anion radicals *in vitro*. Experiments were carried out following a modified protocol previously described (Paya *et al*., 1992). Briefly, superoxide anions were generated by preparing a mixture of hypoxanthine and xanthine oxidase. Reaction mixtures of 1 ml contained the following: K_2HPO_4 (7.96 mM), $NaPO_4H_2$ (2.044 mM) (pH = 7.4), EDTA (1 mM), hypoxanthine (100 μM) and nitro blue tetrazolium (100 μM). Reaction was started by adding 0.0066 u xanthine oxidase (freshly diluted in 100 μl of the above-mentioned buffer), and the rate of nitro blue tetrazolium reduction was measured at 550 nm in a recording spectrophotometer at 25°C. The results are expressed as percentage inhibition of nitro blue tetrazolium reduction.

Drugs

The following drugs were used: calcium dobesilate was a generous gift from Laboratorios Esteve, noradrenaline bitartrate (Sigma), acetylcholine chloride (Sigma), N^G-nitro-L-arginine methyl ester (L-NAME) (Sigma), L-arginine (Sigma), indomethacin (Sigma), 8Br-cyclic GMP (Sigma) hypoxanthine (Sigma), xanthine oxidase (Sigma, specific activity 0.43 u mg^{-1} protein), nitro blue tetrazolium (Sigma), ethylenedinitrilo tetraacetic acid disodium salt dihydrate (EDTA, Merck) and 1H-(1,2,4,)oxadiazolo(4,3-a)quinoxaline-1-one (ODQ) were obtained from Tocris Cookson (Bristol, U.K.). Stock solutions (10^{-2} M) of ODQ and indomethacin were prepared by dissolving them in dimethylsulphoxide (DMSO) 50% and ethanol 50%, respectively. The rest of the drugs were dissolved in deionized water; working solutions were made in Godfraind solution. The concentrations for each chemical or drug are expressed as final concentrations in the chamber in terms of the salt.

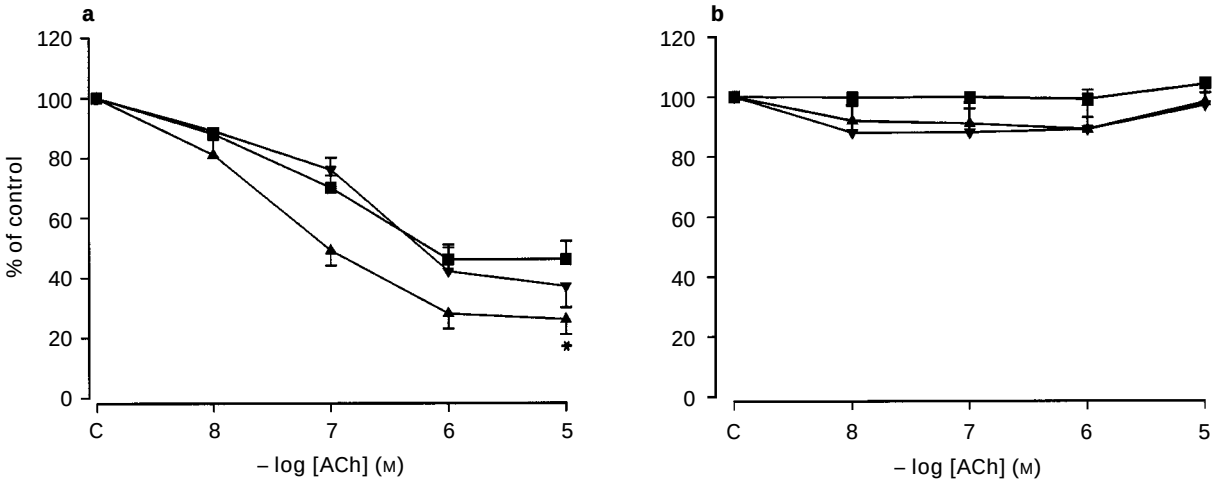

Figure 1 Effect of DOBE on endothelium-dependent relaxation induced by ACh (10^{-8}–10^{-5} M) in arteries with endothelium (+E, a) and arteries without endothelium (−E, b). (■) Control, (▲) DOBE 10^{-5} M, (▼) DOBE 10^{-4} M. Each point shows the mean and vertical lines s.e.mean of 7–9 experiments. *$P < 0.05$.

Statistical analyses

The results are expressed throughout as mean ± s.e.mean of 7–9 rabbits in each group. Concentration-response curves were used to determine the concentration of DOBE producing 50% inhibition of the maximal contractile response (IC_{50}), by use of linear regression analysis over the response range of 20–80% of the maximal inhibition. All protocols concerning animals were approved by the Complutense University of Madrid (EEC official registration 28079-15ABC). Comparisons between the different groups were performed by Student's *t* test for unpaired data and differences were considered significant when $P<0.05$.

Results

Effect of calcium dobesilate on endothelium-dependent relaxation

In arteries with intact endothelium and precontracted with NA 10^{-6} M, ACh 10^{-8}–10^{-5} M caused relaxation in a concentration-dependent manner. The maximal relaxation induced by ACh 10^{-5} M was 54.1 ± 6.2% of control. After incubation with DOBE 10^{-5} M, the endothelium-dependent relaxation induced by ACh increased to 74.2 ± 5.1% ($P<0.05$, $n=10$) (Figure 1a).

On the other hand, in endothelium-denuded arteries ACh was not able to induce relaxation when the arteries were precontracted with NA 10^{-6} M. Furthermore, we found relaxing effects induced by ACh even when the rings were incubated with DOBE 10^{-5} M or 10^{-4} M (Figure 1b).

Effect of calcium dobesilate on NO synthesis

We studied the effect of DOBE on the synthesis of NO (EDRF) (Figure 2). The endothelium-dependent relaxation increased when the arteries were incubated with DOBE (Figure 1a). However, when we pre-incubated the arteries with DOBE plus L-NAME (10^{-8}–10^{-4} M), the effect of DOBE was reverted and the concentration-response curve to ACh was shifted upwards and to the left. The maximal relaxation induced by ACh in the presence of DOBE 10^{-5} M alone was 74.2 ± 5.1% when different concentrations of L-NAME were added as well, the maximal relaxations at the higher concentration used (10^{-4} M) was 22.3 ± 2.4% ($P<0.001$, $n=6$). In addition, when the arteries were incubated in the presence of the substrate of NO synthase, L-Arg (10^{-8}–10^{-4} M) the effect

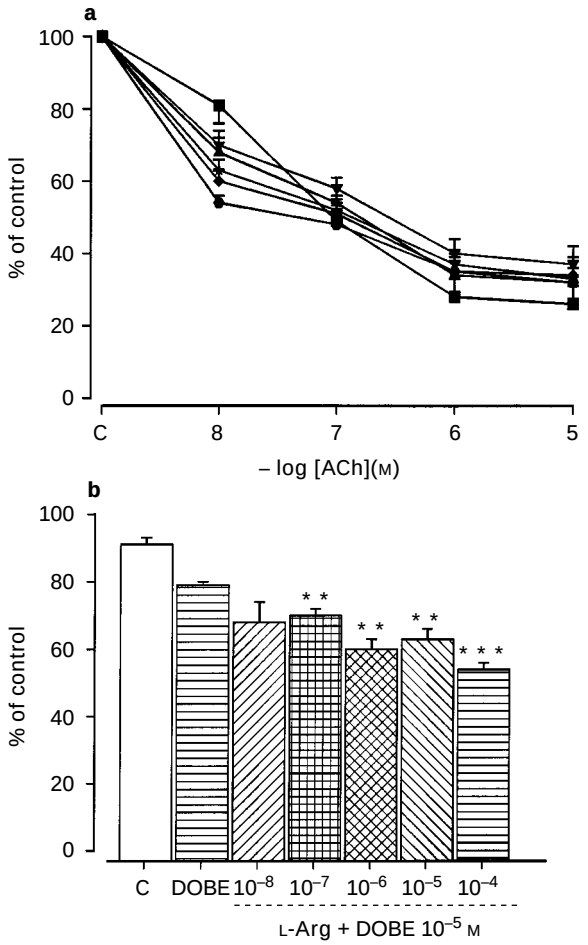

Figure 3 Effect of addition of increasing concentrations of L-arginine (10^{-8}–10^{-4} M) on endothelium-dependent relaxation induced by ACh (in the presence of DOBE 10^{-5} M) (a) (■) Control DOBE 10^{-5} M, (▲) + L-Arg 10^{-8} M, (▼) + L-Arg 10^{-7} M, (◆) + L-Arg 10^{-6} M, (★) + L-Arg 10^{-5} M, (●) + L-Arg 10^{-4} M. Each point shows the mean and vertical lines s.e.mean of 7–9 experiments. (b) The same results depicted in a histogram. $^{**}P<0.01$, $^{***}P<0.001$.

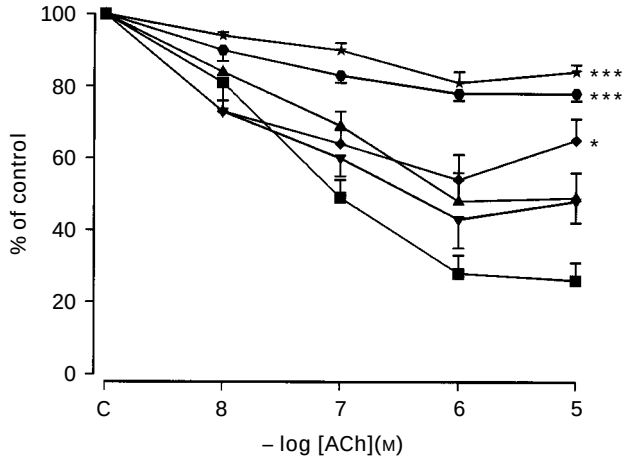

Figure 2 Effect of addition of increasing concentrations of L-NAME (10^{-8}–10^{-4} M) on endothelium-dependent relaxation induced by ACh (in the presence of DOBE 10^{-5} M). (■) Control DOBE 10^{-5} M, (▲) + L-NAME 10^{-8} M, (▼) + L-NAME 10^{-7} M, (◆) + L-NAME 10^{-6} M, (★) + L-NAME 10^{-5} M, (●) + L-NAME 10^{-4} M. Each point shows the mean and vertical lines s.e.mean of 7–9 experiments. $^{*}P<0.05$, $^{***}P<0.0001$.

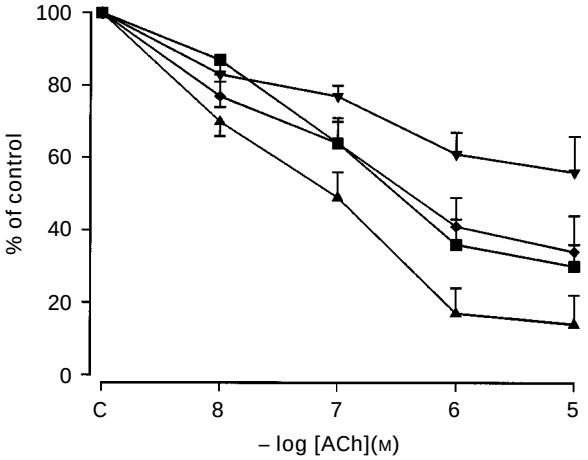

Figure 4 Effect of addition of L-NAME or L-NAME plus L-arginine on endothelium-dependent relaxation induced by ACh (in the presence of DOBE 10^{-5} M). (■) Control, (▲) DOBE 10^{-5} M, (▼) L-NAME 10^{-6} M + DOBE 10^{-5} M, (◆) L-NAME 10^{-6} M + DOBE 10^{-5} M + L-Arg 10^{-4} M. Each point shows the mean and vertical lines s.e.mean of 7–9 experiments.

of DOBE on endothelium-dependent relaxation was increased (Figure 3). L-Arg ($10^{-8}-10^{-4}$ M) plus DOBE 10^{-5} M induced a significant increase in the relaxant response only when the relaxation was induced by ACh 10^{-8} M. Thus, L-Arg 10^{-8} M plus DOBE increased the relaxation induced by ACh 10^{-8} M from $21.4\pm1.2\%$ (in control DOBE) to $32.3\pm6.4\%$ and to $46.2\pm2.4\%$ (L-Arg 10^{-4} M) ($P<0.001$, $n=6$).

L-NAME 10^{-6} M plus DOBE 10^{-5} M caused a decrease in endothelium-dependent relaxation induced by ACh plus DOBE 10^{-5} M. The maximal relaxation (see above) decreased from $82.4\pm7.4\%$ to $42.1\pm7.2\%$ in arteries incubated with DOBE 10^{-5} M and L-NAME 10^{-6} M plus DOBE 10^{-5} M, respectively. L-Arg was able to reverse this inhibition and the maximal relaxation induced by ACh in the presence of DOBE (10^{-5} M) plus L-NAME (10^{-6} M) plus L-Arg (10^{-4} M) was $69.1\pm8.2\%$, as shown in Figure 4.

Effects of calcium dobesilate on synthesis and release of EDHF

In order to test the effect of DOBE on the synthesis and release of EDHF, the arteries were incubated with L-NAME 10^{-4} M and indomethacin 10^{-6} M to block NO and prostacyclin synthesis, respectively. Endothelium-dependent relaxation was then induced by adding ACh ($10^{-8}-10^{-5}$ M) to precontracted rings (Figure 5).

The endothelium-dependent relaxation decreased when the arteries were incubated with L-NAME (10^{-4} M) plus indomethacin (10^{-6} M). The maximal relaxation was $51.2\pm5.8\%$ and $15.5\pm2.3\%$ ($P<0.001$, $n=8$) in the control arteries and those incubated with L-NAME plus indomethacin, respectively. DOBE 10^{-5} M increased, but not significantly, the maximal relaxation, in the presence of L-NAME plus indomethacin from $15.1\pm2.2\%$ to $24.2\pm4.3\%$.

Effect of calcium dobesilate on stimulation of soluble guanylate cyclase by NO

In the other group of experiments, the endothelium-dependent relaxation induced by ACh was performed in the presence of an antagonist of soluble guanylyl cyclase, ODQ, and in the presence of a permeable analogue of cyclic GMP, 8Br-cyclic GMP,

ODQ 10^{-6} M reduced the endothelium-dependent relaxation induced by ACh ($10^{-8}-10^{-5}$ M). The maximal relaxation induced by ACh 10^{-5} M decreased from $53.3\pm4.2\%$ to $32.4\pm6.6\%$ in the presence of ODQ. When the aortic rings were incubated with both ODQ and DOBE 10^{-5} M the endothelium-dependent relaxation induced by ACh 10^{-5} M increased from $32.4\pm6.6\%$ to $49.3\pm5.2\%$ in ODQ-incubated arteries and ODQ plus DOBE-incubated arteries, respectively (data not shown).

We also tested the effects of DOBE on endothelium-dependent relaxation in the presence of the permeable analogue of cyclic GMP, 8Br-cyclic GMP ($10^{-8}-10^{-4}$ M). As shown in Figure 6, only 8Br-cyclic GMP 10^{-4} M (the highest concentration used) increased the endothelium-dependent relaxation induced by ACh ($10^{-8}-10^{-5}$ M) in the presence of DOBE 10^{-5} M. The IC_{50} for ACh decreased in arteries incubated with 8Br-cyclic GMP 10^{-4} M plus DOBE 10^{-5} M from $5\pm0.5\times10^{-7}$ M (control DOBE) to $5.1\pm0.4\times10^{-8}$ M. However, the maximal relaxation induced by ACh 10^{-5} M did not change when the arteries were incubated with 8Br-cyclic GMP plus DOBE.

Scavenger effect of DOBE on superoxide anion radicals

We tested the ability of calcium dobesilate to scavenge superoxide anion radicals *in vitro*, by use of the hypoxanthine/

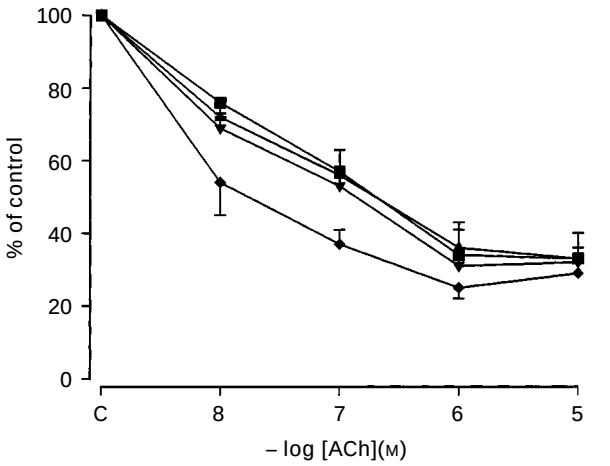

Figure 6 Effect of increasing concentration of 8Br cyclic GMP ($10^{-8}-10^{-4}$ M) on endothelium-dependent relaxation induced by ACh (in the presence of DOBE 10^{-5} M). (■) Control DOBE 10^{-5} M, (▲) 8Br cyclic GMP 10^{-8} M + DOBE 10^{-5} M, (▼) 8Br cyclic GMP 10^{-6} M + DOBE 10^{-5} M; (◆) 8Br cyclic GMP 10^{-4} M + DOBE 10^{-5} M. Each point shows the mean and vertical lines indicate s.e.mean of 7–9 experiments.

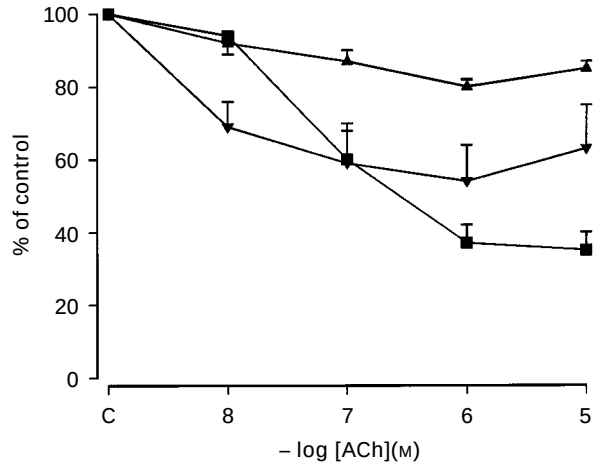

Figure 5 Effects of DOBE on synthesis/release of EDHF. EDRF and prostacyclin synthesis were inhibited by preincubating the arteries with both L-NAME 10^{-4} M and indomethacin 10^{-6} M. (■) Control, (▲) L-NAME 10^{-4} M + indomethacin 10^{-6} M, (▼) L-NAME 10^{-4} M + indomethacin 10^{-6} M + DOBE 10^{-5} M. Each point shows the mean and vertical lines s.e.mean of 7–9 experiments.

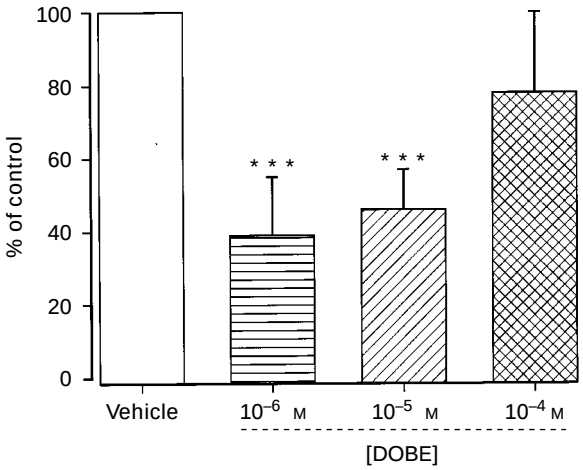

Figure 7 Scavenger effect of DOBE. Each column shows the percentage inhibition of the nitro blue tetrazolium reduction (triplicated experiments). ***$P<0.001$.

xanthine oxidase reaction. DOBE 10^{-6} M decreased the rate of reduction of nitro blue tetrazolium to $39.1 \pm 16.2\%$ ($P<0.001$) of control. DOBE 10^{-5} M also decreased the rate of reduction to $46.3 \pm 11.4\%$ ($P<0.001$) of control, while the highest dose of DOBE used did not show any scavenging effect, as shown in Figure 7. The concentrations of DOBE shown to be effective in these studies are in the range of the plasma levels found after the common rabbit and human oral dose of 500 mg (plasma concentration 8 μg ml^{-1} equivalent to 2×10^{-5} M (Benakis *et al*., 1974).

Discussion

In this paper we have presented evidence that calcium dobesilate, a drug commonly used to prevent vascular disorders in diabetes mellitus, enhances the endothelium-dependent relaxation induced by ACh. This effect is clearly endothelium-dependent because when the endothelium was removed, the effect disappeared (Figure 3b). It seems likely that DOBE acts on the endothelium-dependent relaxing factor, EDRF (NO).

As shown in Figure 2, the effect of DOBE was inhibited when the rings were incubated with increasing concentrations of the NO-synthase inhibitor, L-NAME, and this effect was reversed by L-Arg, the substrate in NO synthesis. This does not mean that DOBE acts directly on NO-synthase. NO synthesis is a complex mechanism not fully understood. The process, like others in cells, seems to be ruled by $[Ca^{2+}]_i$ (Peach *et al*., 1987). Agonist stimulation of endothelial cells induces calcium release from intracellular stores. This is followed by an influx of external calcium to maintain an increased level of cytosolic calcium (Colden-Standfield *et al*., 1987; Hallan *et al*., 1988). Membrane hyperpolarization in endothelial cells is induced as a consequence of activation of calcium-dependent potassium channels by an increase in cellular calcium (Busse *et al*., 1988). In addition, a N-terminal myristoylation of NO-synthase (Busconi & Michel, 1993) has been observed and it is plausible that the regulation of endothelial NO may affect the biological activity of this enzyme system.

Calcium dobesilate, at first, might act on any of these steps in the NO synthesis, although an action of potassium channels could be implicated. We have previously described the relaxation induced by DOBE on a maintained contraction induced by NA or KCl, whereas DOBE was less effective when the arteries were contracted with KCl 80 mM (Ruiz *et al*., 1995), which means that there is an extracellular potassium overload. This fact could also implicate DOBE in the hyperpolarization of vascular smooth muscle cells by NO (Archer *et al*., 1994). This is unlikely, since the effects of DOBE disappeared when the endothelium was removed.

In another group of experiments we tested the effect of DOBE on synthesis/release of EDHF. As shown in Figure 5, DOBE did not significantly increase the endothelium-dependent relaxation that remained when we blocked both NO and prostacyclin synthesis with L-NAME and indomethacin, respectively. This remainding relaxation could be attributed to the action of EDHF.

Previous studies have indicated that some of the cardiovascular disorders associated with diabetes mellitus are a consequence of alterations in the reactivity of blood vessels (Weidman *et al*., 1979). In preliminary data, abnormal endothelium-dependent relaxation to acetylcholine has been described in some animal models (Durante *et al*., 1988; Rösen *et al*., 1995) as well as in diabetic subjects (de Tejada *et al*., 1989; McNally *et al*., 1992; McVeigh *et al*., 1992). Furthermore, the biological availability of NO might be reduced in diabetic states, since it is rapidly quenched by reactive oxygen radicals or by advanced glycation end-products (Pieper & Gross, 1988; Bucala *et al*., 1991). Among the mechanisms that have been proposed to explain diabetic vascular dysfunction, underlying hyperglycaemia is being being viewed as increasingly central to its aetiology. Several explanations have been put forward which might associate hyperglycaemia with reduced endothelium-dependent relaxation. These include a role for the glucose-stimulated increase in polyol pathway activity and the redox changes associated with increased sorbitol flux (Williamson *et al*., 1990; Cameron & Cotter, 1992).

However, calcium dobesilate showed a scavenger effect on superoxide anion radicals (as shown Figure 7) generated by the hypoxanthine/xanthine oxidase reaction. This effect could be an additional mechanism implicated in the action of calcium dobesilate on the vascular complication that occurs in diabetes mellitus. Previous studies have shown that endothelium-dependent relaxation of diabetic vasculature is more sensitive to free radical-induced injury (Pieper & Gross, 1988). The abnormal endothelium-dependent relaxation in aorta from diabetic rabbits was restored to normal by superoxide dismutase (SOD). This suggests a role for superoxide anions in the endothelial cell abnormality caused by diabetes mellitus (Tesfamariam & Cohen, 1992). In addition, aortae from both normal and diabetic rabbits exposed to elevated concentrations of glucose *in vitro* demonstrate impairment of endothelium-dependent relaxation (Oyama *et al*., 1986; Wohaieb & Dean, 1987; Wolff & Dean, 1987; Tesfamariam *et al*., 1989).

Taken together, these results provide evidence that calcium dobesilate is able to reduce the vascular disorders associated with diabetes mellitus since it enhances the synthesis/release of NO (spontaneous as well as stimulated) and prevents the destruction of endothelium-dependent relaxation by superoxide anion radicals.

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