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Gender differences and antioxidant treatment affect aortic reactivity in short-term diabetic rats

Christian Pinna*, Andrea Cignarella, Rossella Zanardo, Chiara Bolego, Lina Puglisi

Department of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy

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Abstract

Diabetes is associated with gender-specific macrovascular complications arising from increased oxidant stress in the vascular wall. In this study, male and female rats were treated with two structurally unrelated drugs sharing antioxidant properties, lercanidipine and Leucoselect™ (both 3 mg/kg/day), for 1 week starting 1 day after streptozotocin-diabetes induction. Concentration–response curves to L-nitroarginine methylester (L-NAME), superoxide dismutase and acetylcholine in aortic rings showed significantly greater nitric oxide-mediated relaxation in female compared with male non-diabetic rats. Diabetes increased contractility to noradrenaline and L-NAME in both genders, whereas relaxation to acetylcholine and iloprost were significantly attenuated in females only. Treatment with lercanidipine and Leucoselect restored, at least in part, responses to noradrenaline, acetylcholine and iloprost without affecting those to L-NAME and sodium nitroprusside. Unexpectedly, both drugs impaired superoxide dismutase response in female tissues. In conclusion, female rat aorta is markedly exposed to short-term diabetic vascular injury, which may be prevented by antioxidant treatment.

Keywords: Diabetes; Aorta; Nitric oxide (NO); Lercanidipine; Leucoselect; Antioxidant

1. Introduction

Recently, the endothelium has emerged as the tissue of first-line defence against vascular disease. Several studies have demonstrated the antiatherosclerotic properties of endothelium-derived nitric oxide (NO) (Hayashi et al., 2000; Marano et al., 1999; Bult et al., 1999). There is evidence that endothelium-dependent vasodilation is impaired in diabetes mellitus. This has been related to increased vascular production of oxygen free radicals, reduced antioxidant activity and hyperglycaemia, eventually leading to decreased endothelial NO and prostacyclin production (Fedele and Giugliano, 1997; Öztürk et al., 1996; Tesfamariam, 1994). On the other hand, the great amount of peroxynitrite produced following activation of the inducible isoform of NO synthase (iNOS) has been implicated in the pathophysiology of several vascular disorders and of diabetes itself (Marin and Rodríguez-Martínez, 1997; Ross, 1999).

Diabetes mellitus is an independent risk factor for cardiovascular disease, including atherosclerosis and microangiopathy. Clinical and epidemiological studies indicate that female patients with diabetes have particularly poor outcomes after clinical cardiovascular events (Garcia et al., 1974; Barrett-Connor and Bush, 1991). Therefore, the vascular protective effects of female hormones appear to be attenuated in diabetes (Sowers, 1998; Gaba et al., 1999). In addition, possible differences exist between the two diabetes types (insulin- and non-insulin-dependent) as cardiovascular risk factors. For instance, dyslipidemia is far more prevalent in the latter than in the former type, which may lead to different clinical manifestation. Furthermore, apart from the maintenance of near-normal glycaemic values during the entire period of diabetes, currently available drugs show little effect on other complications after the onset of diabetes. However, animal studies indicated that administration of a number of drugs such as aldose reductase inhibitors, gangliosides, ω-6-essential fatty acids and antioxidants may be effective in preventing the onset of diabetes neuropathy (Fedele and Giugliano, 1997). In addition, epidemiological studies have shown that antioxidant intake reduced the specific risk of

* Corresponding author. Tel.: +39-025-835-8304; fax: +39-025-835-8284.

E-mail address: Christian.Pinna@unimi.it (C. Pinna).

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cardiovascular disease and atherosclerosis (Diaz et al., 1997; Ness and Powles, 1997; Herlog et al., 1993; Stampfer et al., 1993).

With this in mind, we set out to understand whether (a) antioxidant treatment protects the vascular wall from functional impairment during the early phase of diabetes development and (b) the vascular effects of antioxidants in diabetic rats are gender-specific. For this purpose, we selected two drugs that are structurally unrelated but share antioxidant properties, namely the Ca^{2+} channel antagonist lercanidipine and the phospholipid-procyanidins complex from grape seed Leucoselect™. Among Ca^{2+} channel antagonists, lercanidipine is endowed with the greatest vascular selectivity not associated with cardiac effects (Angelico et al., 1999; Guarnieri et al., 1996) and shows antioxidant properties in vivo (Digiesi et al., 2000; Cesarone et al., 2000). In addition, lercanidipine has antiproliferative effects that may interfere with events involved in atherogenesis (Corsini et al., 1996; Soma et al., 1998). On the other hand, Leucoselect is a mixture of polyphenols extracted and purified from grape seeds (Gabetta et al., 2000), whose antioxidant effect has been demonstrated in both animals (Ursini et al., 1999) and humans (Nuttall et al., 1998). After drug treatment, vascular reactivity was measured in the isolated thoracic aorta from both male and female animals by isometric tension recording following challenge with different vasoactive compounds.

2. Materials and methods

2.1. Animals

Experiments were performed on isolated aortic rings excised from male and female Sprague–Dawley rats weighing 200–225 g. Animals were divided into eight groups as follows: (a) non-diabetic males; (b) untreated diabetic males; (c) lercanidipine-treated diabetic males; (d) Leucoselect-treated diabetic males; (e) non-diabetic females; (f) untreated diabetic females; (g) lercanidipine-treated diabetic females; (h) Leucoselect-treated diabetic females. Insulin-dependent diabetes was induced where indicated by a single i.v. injection of streptozotocin (60 mg/kg) dissolved in 0.1 N citrate buffer (pH 4.5). Control animals were injected with vehicle only. Diabetes induction was considered successful when glucose levels were higher than 16 mmol/l (Glucotrend, Roche Diagnostics, Monza, Italy). This was generally the case within 72 h after streptozotocin treatment. Lercanidipine and Leucoselect were both administered p.o. at a concentration of 3 mg/kg/day for 7 days, in agreement with previous studies (Soma et al., 1998; Nuttall et al., 1998), starting 24 h after diabetes induction. Experiments were carried out the day after last treatment. Procedures involving animals and their care were conducted in compliance with local institutional guidelines from the University of Milan.

2.2. Ex-vivo experiments on isolated aortic rings

Thoracic aortas were carefully excised, cleaned of fat and connective tissue, and cut into 5-mm rings. Tissues were then prepared for isometric tension recording as previously described (Bolego et al., 1997, 1999). The aortic rings were set up in 5-ml organ baths and perfused with Krebs solution at 37 °C continuously bubbled with 95% O_2: 5% CO_2. The Krebs' solution contained (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.1, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 5.5; pH 7.4. Vascular tissues were then connected to force transducers for isometric tension recording (two-channel recorder Gemini 7070, Basile, Comerio, Italy). Thirty minutes after mounting in the organ bath under a resting tension of 1.5 g (14.7 mN), tissues were challenged with a submaximal concentration of noradrenaline (10^{-6} M) to check the vitality of preparations and then washed with fresh Krebs' solution. The equilibration period was allowed to continue for another 30 min. Different vasoactive compounds were then added cumulatively to the preparations. With the exception of noradrenaline itself, concentration–response curves for such compounds were performed after precontracting tissues with noradrenaline (EC_{50}). If preparations were used at resting tone, evoked relaxant responses would often be too slight to calculate EC_{50} and maximal response. Concentration–response curves to noradrenaline were expressed in mN/mg tissue, whereas contractile or relaxant responses to other agents were expressed as percentage of noradrenaline-induced contraction or percentage of relaxation of noradrenaline-precontracted tissues, respectively.

2.3. Drugs and chemicals

Noradrenaline hydrochloride, nitro-L-arginine methyl ester (L-NAME), superoxide dismutase, acetylcholine, sodium nitroprusside and streptozotocin were purchased from Sigma. Iloprost (SHL 401) was purchased from Schering (Berlin, Germany). Recordati and Indena (both Milan, Italy) kindly provided lercanidipine and Leucoselect, respectively. All compounds were freshly dissolved in distilled H_2O except lercanidipine, which was dissolved in polyethylene glycol/H_2O (50:50).

2.4. Statistical analysis

All data were expressed as mean ± S.E.M. of eight experiments and represent unpaired data. Concentration–response curves were calculated by the software Prism and compared by means of analysis of variance (ANOVA); when P values were less than 0.05, the treatment affected

Table 1
Values of sensitivity to each vasoactive compound as expressed by pD_2 in experimental groups

Group	Acetylcholine	Superoxide dismutase	L-NAME	Sodium nitroprusside	Iloprost	Noradrenaline
Males						
Control	6.8 ± 0.05	0.3 ± 0.16	4.5 ± 0.05	8.7 ± 0.05	9.8 ± 0.16[a]	7.5 ± 0.19
Diabetic	6.8 ± 0.05	0.3 ± 0.14	4.6 ± 0.01	8.4 ± 0.03	10 ± 0.10	7.4 ± 0.05
Diabetic + Leucoselect	6.6 ± 0.03	0.3 ± 0.11	4.7 ± 0.01	8.3 ± 0.08	10 ± 0.07	8.1 ± 0.38[c]
Diabetic + lercanidipine	7.0 ± 0.09	0.3 ± 0.13	4.8 ± 0.03	8.2 ± 0.02	10 ± 0.07	7.5 ± 0.13
Females						
Control	6.9 ± 0.05	0.3 ± 0.26	4.6 ± 0.05	8.9 ± 0.06	6.9 ± 0.09	7.7 ± 0.07
Diabetic	6.5 ± 0.07	0.3 ± 0.28	4.5 ± 0.05	8.9 ± 0.09	9.0 ± 0.21[b]	7.8 ± 0.11
Diabetic + Leucoselect	6.9 ± 0.04	0.4 ± 0.20	4.6 ± 0.01	9.4 ± 0.20	8.0 ± 0.30[c]	8.1 ± 0.19
Diabetic + lercanidipine	7.2 ± 0.03	0.5 ± 0.19	4.6 ± 0.05	9.4 ± 0.08	8.0 ± 0.25[c]	7.8 ± 0.02

Data are expressed as pD_2 values ± S.E.M. for all concentration–response curves, except for concentration-related responses to superoxide dismutase where slope instead of pD_2 value is shown.
[a] Statistical significance: $P < 0.05$ (vs. female).
[b] Statistical significance: $P < 0.05$ (vs. non diabetic, same gender).
[c] Statistical significance: $P < 0.05$ (vs. untreated diabetic, same gender).

the response over the tested range of concentration (Ludbrook, 1994). In addition, pD_2 values and maximal responses (E_{max}) for each agonist were compared by one-way ANOVA followed by Tukey–Kramer post hoc test.

3. Results

3.1. Serum glucose levels

A sixfold increase in glycaemia values compared with controls was observed in untreated diabetic rats 8 days following streptozotocin injection (males: 32 ± 1 vs. 5 ± 0.3 mmol/l, females: 33 ± 2 vs. 5 ± 0.5 mmol/l; $n = 8$ for both groups). After treatment with both lercanidipine and Leucoselect, neither body weight (data not shown) nor glycaemia differed significantly as compared with untreated diabetic rats (males: 33 ± 1 and 32 ± 1 mmol/l, respectively; females: 33 ± 1 and 33 ± 0.9 mmol/l, respectively; $n = 8$).

3.2. Sensitivity and maximal responses to agonists

Concentration–response curves were obtained for several agonists in aortic rings excised from non-diabetic and from untreated, Leucoselect- and lercanidipine-treated streptozotocin-diabetic male and female rats. Table 1 shows sensitivity values (pD_2 ± S.E.M.) for all concentration–

Table 2
Maximal response (E_{max}) to each vasoactive compound in experimental groups

Group	Acetylcholine	Superoxide dismutase	L-NAME	Sodium nitroprusside	Iloprost	Noradrenaline
Males						
Control	16 ± 6.0	43 ± 7.3[a]	118 ± 3.9[a]	0 ± 0.3	23 ± 3.9[a]	1.60 ± 0.30
Diabetic	26 ± 3.1	52 ± 8.8	158 ± 13[b]	5 ± 1.5	22 ± 4.8	2.30 ± 0.35
Diabetic + Leucoselect	22 ± 7.0	56 ± 5.5	166 ± 11	0 ± 6.0	22 ± 4.6	1.79 ± 0.36
Diabetic + lercanidipine	9 ± 5.8	47 ± 4.8	142 ± 10	0 ± 1.0	33 ± 5.4	1.52 ± 0.26
Females						
Control	8 ± 4.0	29 ± 6.0	130 ± 3.9	0 ± 3.4	44 ± 2.6	1.36 ± 0.20
Diabetic	14 ± 6.2	25 ± 4.9	155 ± 10	0 ± 5.6	29 ± 1.4	2.30 ± 0.30[b]
Diabetic + Leucoselect	10 ± 6.0	19 ± 6.1	156 ± 11	0 ± 7.3	46 ± 3.9	1.70 ± 0.10
Diabetic + lercanidipine	4 ± 4.0	28 ± 2.9	146 ± 10	0 ± 3.1	59 ± 6.6[c]	1.40 ± 0.20[c]

Data are expressed as E_{max} ± S.E.M. for all concentration–response curves. With the exception of noradrenaline itself, all data are presented as percent of noradrenaline precontration. E_{max} for noradrenaline is expressed in mN/mg tissue.
[a] Statistic significance: $P < 0.05$ (vs. female).
[b] Statistic significance: $P < 0.05$ (vs. non diabetic, same gender).
[c] Statistic significance: $P < 0.05$ (vs. untreated diabetic, same gender).

response curves. For superoxide dismutase-induced responses, curve slope instead of pD_2 is shown. pD_2 values for all agonists were not significantly different among groups except for iloprost (see Section 3.6) and noradrenaline (Section 3.3). Maximal responses ($E_{max} \pm$ S.E.M.) to all agonists are shown in Table 2.

3.3. Noradrenaline-mediated vascular contractility

Contractile responses to noradrenaline (3×10^{-10}–10^{-5} M) in aortic rings from both healthy male and female rats were comparable (Fig. 1A and B). The response curves to noradrenaline in tissues from untreated diabetic animals showed significantly greater contractility in both genders ($P < 0.001$) as compared with controls based upon analysis of variance of the curves. The response curves of untreated diabetic male and female aorta were not significantly different (Fig. 1A and B). In diabetic females, E_{max} was also significantly greater than that seen in tissues from control females (Fig. 1B, Table 2). Lercanidipine treatment completely recovered contractile responses in aortic preparations from both male (Fig. 1A) and female (Fig. 1B) diabetic rats. In fact, the response curves seen in tissues from lercanidipine-treated and non-diabetic animals of both genders were comparable (two-way ANOVA, $P = 0.66$ and 0.97, respectively). E_{max} was significantly decreased by about 40% (Table 2). In contrast, Leucoselect treatment only partially recovered noradrenaline contraction in the aorta of female (Fig. 1B; $P < 0.05$), but not in that of male diabetic animals ($P > 0.05$). In fact, the response curve seen in tissues from Leucoselect-treated female animals was significantly different from that of both non-diabetic ($P < 0.01$) and untreated diabetic animals ($P < 0.01$). Leucoselect treatment also increased sensitivity to noradrenaline in preparations from diabetic male rats (Table 1).

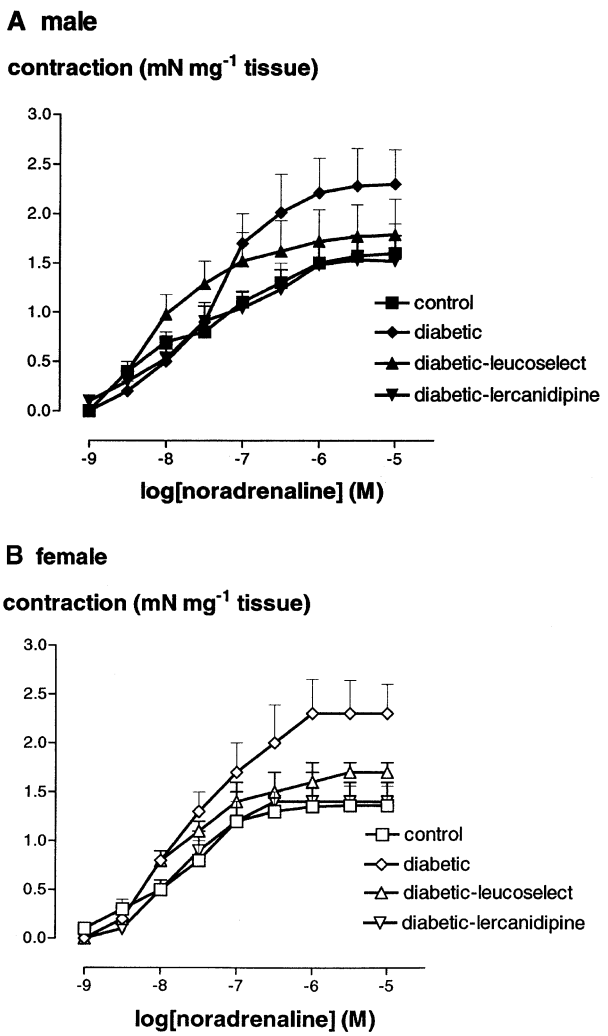

Fig. 1. Cumulative concentration–response curves for noradrenaline in aortic preparations obtained from control, diabetic, lercanidipine-treated diabetic and Leucoselect-treated diabetic male (A) and female (B) rats. Contractile tension was expressed as mN/mg weight tissue. Data are shown as means ± S.E.M. ($n = 8$ in each group). Concentration–response curves differ significantly as follows: $P < 0.001$ control vs. diabetes, irrespective of gender; $P < 0.001$ lercanidipine-treated vs. untreated diabetic, irrespective of gender ; $P < 0.05$, Leucoselect-treated vs. untreated diabetic females (ANOVA).

3.4. Tone-related rate of NO production

Responses to the NO synthase inhibitor L-NAME and to the free radical scavenger superoxide dismutase were evaluated to assess the basal release of NO from rat aorta. The response curves to L-NAME (10^{-6}–10^{-4} M) indicated greater contractility in aortic rings from non-diabetic female compared with male rats ($P < 0.05$), reflecting an increased rate of NO production in precontracted aorta from females. E_{max} value was significantly greater as well ($P < 0.05$, Table 2). The response curves of untreated diabetic male and female aorta were not significantly different (Fig. 2A and B). Diabetes significantly increased ($P < 0.01$) responsiveness to L-NAME in tissues from both male and female animals; in male tissues, E_{max} value was also significantly greater (Table 2). Treatment with either lercanidipine or Leucoselect did not affect aortic responsiveness to L-NAME in both male and female diabetic rats (Fig. 2A and B).

Aorta from non-diabetic female rats showed a significant increase ($P < 0.01$) in superoxide dismutase-induced (10^{-1}–10^{2} U/ml) relaxation, which reflects a higher amount of NO available for degradation, compared with males. Again, this increase could be seen also at the E_{max} level ($P < 0.05$, Table 2). Similarly, the response curve to superoxide dismutase in the aorta from diabetic females was significantly different from that of diabetic males ($P < 0.05$). Diabetes did not significantly alter superoxide dismutase-induced relaxant responses irrespective of animals' gender. In contrast, analysis of response curves showed that lercanidipine and Leucoselect treatment significantly reduced superoxide dismutase-induced relax-

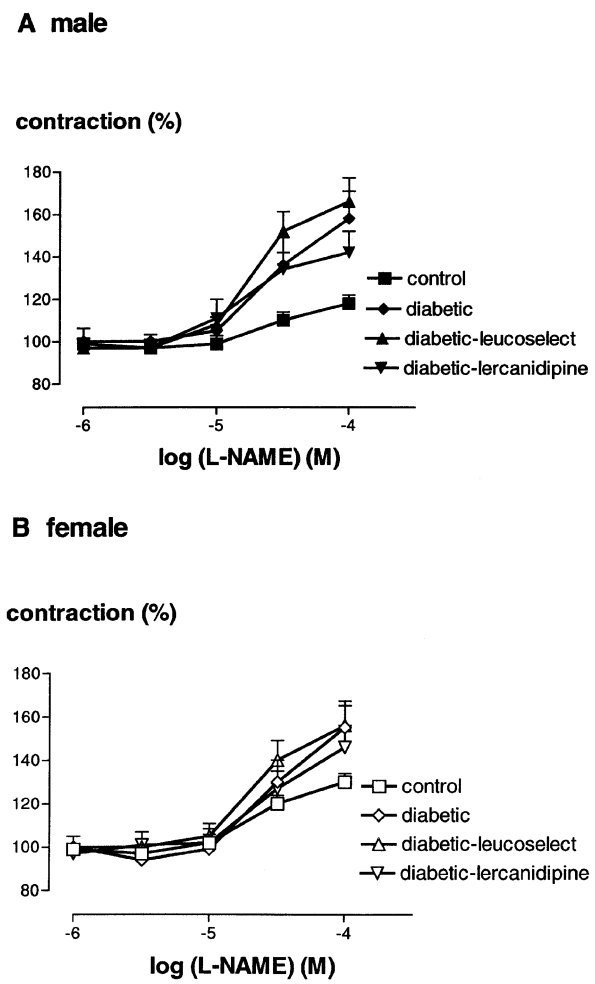

Fig. 2. Cumulative concentration–response curves for nitro-L-arginine methyl ester (L-NAME) in aortic preparations obtained from control, diabetic, lercanidipine-treated diabetic and Leucoselect-treated diabetic male (A) and female (B) rats. Data represent the mean percent of noradrenaline precontraction ± S.E.M. ($n = 8$ in each group). Concentration–response curves differ significantly as follows: $P < 0.05$, control females vs. males; $P < 0.01$, control vs. diabetes, irrespective of gender (ANOVA). Neither pharmacological treatment significantly affected responsiveness to L-NAME.

ation in the aorta from female (ANOVA, $P < 0.01$ for both treatments; Fig. 3B), but not in that from male diabetic rats (Fig. 3A).

3.5. Endothelium-dependent relaxation

The relaxant response evoked by endothelium-dependent vasodilators such as acetylcholine is known to be mediated by NO. In aortic rings from control female rats, relaxant responses to acetylcholine (10^{-9}–3×10^{-5} M) were significantly greater ($P < 0.05$) than in those from male rats (Fig. 4A and B). The response curves of untreated diabetic male and female aorta were not significantly different (Fig. 4A and B). Diabetes significantly impaired ($P < 0.05$) responses to acetylcholine in the aorta from female rats only. Lercanidipine and Leucoselect, however, were able to recover these responses completely in preparations from female rats (Fig. 4B).

3.6. Endothelium-independent relaxation induced by iloprost

The concentration-related curve to the stable prostacyclin analogue iloprost was remarkably shifted to the right, as illustrated from the lower pD_2 value ($P < 0.001$; Table 1, Fig. 5), and revealed an increase in maximal response (Table 2) in tissues from non-diabetic female compared with male rats. Diabetes increased the sensitivity of female

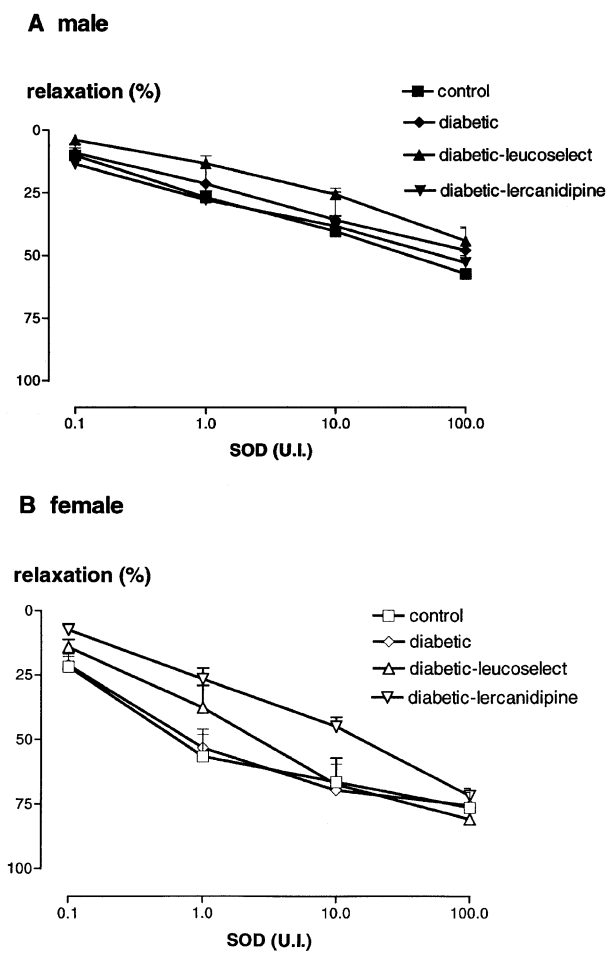

Fig. 3. Cumulative concentration–response curves for superoxide dismutase in aortic preparations obtained from control, diabetic, lercanidipine-treated diabetic and Leucoselect-treated diabetic male (A) and female (B) rats. Data represent the mean percent of relaxation of noradrenaline-precontracted tissues ± S.E.M. ($n = 8$ in each group). Concentration–response curves differ significantly as follows: $P < 0.01$, control male vs. female; $P < 0.05$, untreated diabetic females vs. untreated diabetic males; $P < 0.01$, lercanidipine-treated vs. untreated diabetic females; $P < 0.01$, Leucoselect-treated vs. untreated diabetic females (ANOVA).

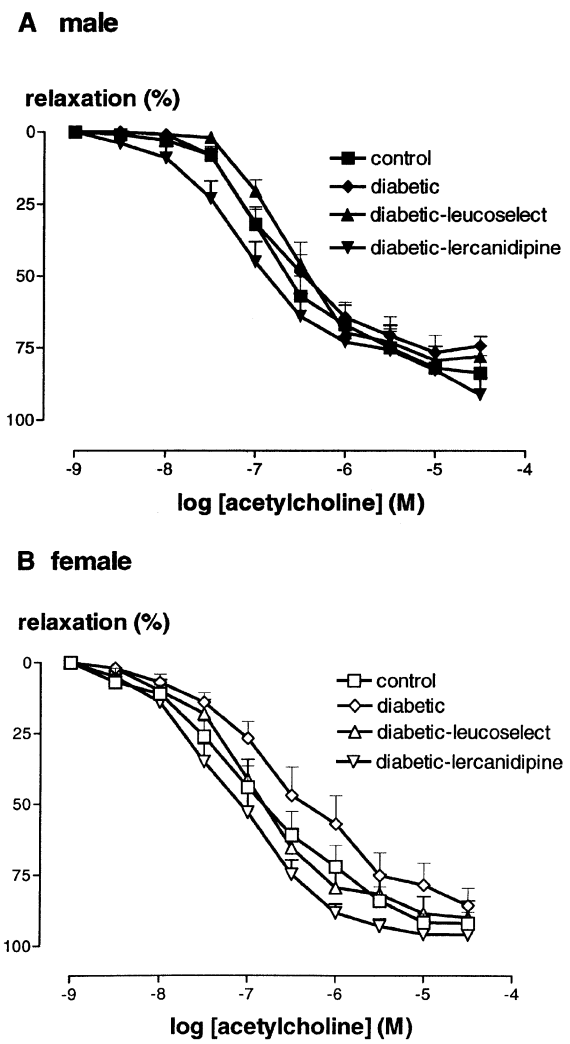

Fig. 4. Cumulative concentration–response curves for acetylcholine in aortic preparations obtained from control, diabetic, lercanidipine-treated diabetic and Leucoselect-treated diabetic male (A) and female (B) rats. Data represent the mean percent of relaxation of noradrenaline-precontracted tissues ± S.E.M. ($n = 8$ in each group). Concentration–response curves differ significantly as follows: $P < 0.05$ control males vs. females; $P < 0.05$ control vs. diabetic females; $P < 0.05$ lercanidipine-treated vs. untreated diabetic females; $P < 0.05$ Leucoselect-treated vs. untreated diabetic females (ANOVA).

but not that of male tissues to iloprost by two orders of magnitude ($P < 0.001$; Table 1). Lercanidipine treatment partially reduced tissue sensitivity to iloprost only in the aorta from female animals (Table 1). The response curve to iloprost seen in lercanidipine-treated females was significantly shifted to the right compared with that of untreated diabetic females (Fig. 5B). Similarly, the response curve to iloprost in tissues from Leucoselect-treated diabetic females indicated a significantly impaired relaxation compared with untreated diabetic females (Fig. 5B), though to a lower extent than the response curves of lercanidipine-treated animals. The sensitivity to iloprost in the aorta from Leucoselect-treated diabetic females was also significantly lower than in that from untreated diabetic females ($P < 0.05$; Table 1).

3.7. Response to exogenous NO donors

Endothelium-independent relaxation elicited by increasing concentrations of the exogenous NO donor sodium nitroprusside (10^{-11}–10^{-7} M) in aortic rings from non-diabetic male and female rats were comparable (Fig. 6A and B). In the aorta of male diabetic rats, the response curve to sodium nitroprusside was significantly different, indicating impaired relaxation, compared with non-diabetic males ($P < 0.001$; Fig. 6A) as well as with diabetic females ($P < 0.001$; Fig. 6A and B). However, no significant

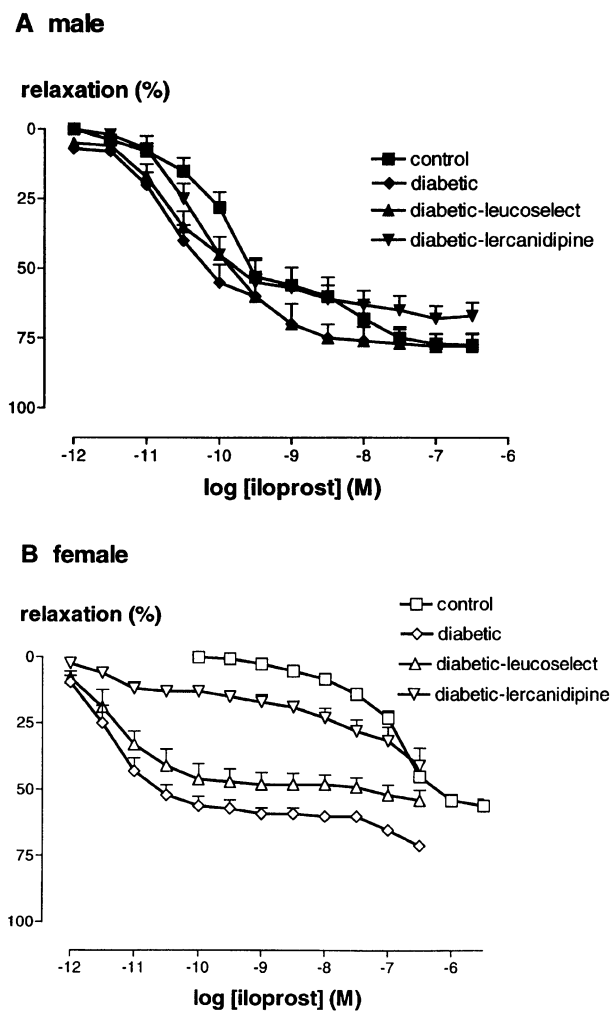

Fig. 5. Cumulative concentration–response curves for iloprost in aortic preparations obtained from control, diabetic, lercanidipine-treated diabetic and Leucoselect-treated diabetic male (A) and female (B) rats. Data represent the mean percent of relaxation of noradrenaline-precontracted tissues ± S.E.M. ($n = 8$ in each group). Concentration–response curves differ significantly as follows: $P < 0.001$ control males vs. females; $P < 0.001$ control vs. diabetic females; $P < 0.01$ lercanidipine-treated vs. untreated diabetic females; $P < 0.01$ Leucoselect-treated vs. untreated diabetic females (ANOVA).

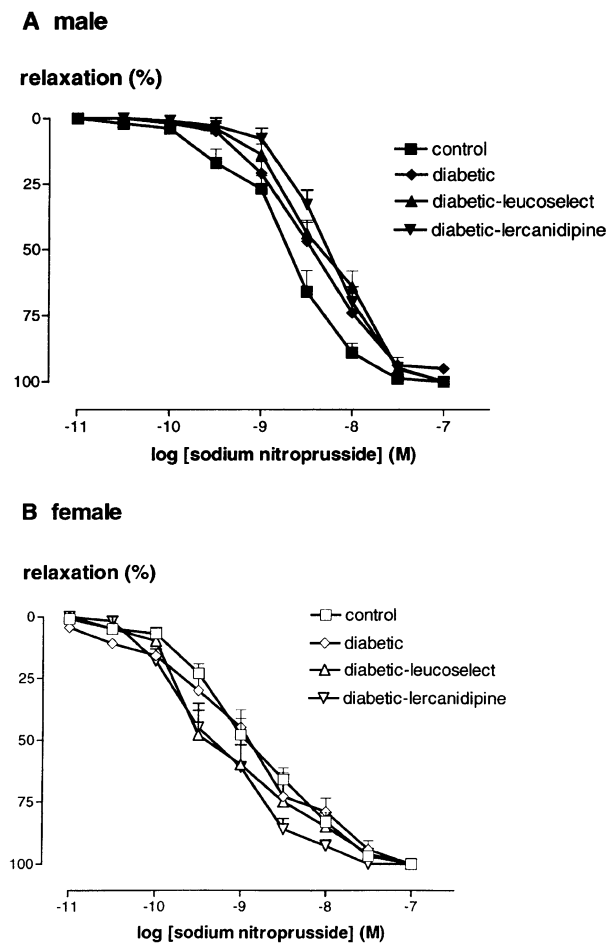

Fig. 6. Cumulative concentration–response curves for sodium nitroprusside in aortic preparations obtained from control, diabetic, lercanidipine-treated diabetic and Leucoselect-treated diabetic male (A) and female (B) rats. Data represent the mean percent of relaxation of noradrenaline-precontracted tissues ± S.E.M. ($n = 8$ in each group). Concentration–response curves differ significantly as follows: $P < 0.001$ untreated diabetic males vs. untreated diabetic females.

change in response to sodium nitroprusside was produced by lercanidipine or Leucoselect treatment irrespective of animals' gender (Fig. 6A and B).

4. Discussion

In the present study, we evaluated the effects of two structurally unrelated compounds with antioxidant activity on responsiveness to several vasoactive agonists in the aorta of streptozotocin-diabetic rats as well as gender differences in response to the same agonists. In agreement with previous studies, significant gender differences were observed in aortic preparations from non-diabetic rats (Hayashi et al., 1992, 1995). In particular, relaxant responses to superoxide dismutase, acetylcholine and iloprost and the contractile response to L-NAME were significantly different in female and male rats, as indicated by analysis of variance of the curves. This results from a greater spontaneous and/or stimulated release of NO from endothelial cells in tissues from female compared with male rats (Hayashi et al., 1992), smooth muscle cell sensitivity to exogenous NO-releasing compounds being unchanged, as indicated by comparable concentration–response curves to sodium nitroprusside (Fig. 6). Conversely, both maximal response and sensitivity to iloprost were impaired in female aorta. To the best of our knowledge, this is the first report of such a gender effect on the response to this stable prostacyclin analogue. Iloprost induces vascular relaxation via endothelium-independent mechanisms by activating IP receptors located on smooth muscle cell membrane (Campbell and Halushka, 1996). Since general consensus from the literature points to a stimulation of prostacyclin release by female sex hormones (Chang et al., 1980; Wakasugi et al., 1989; Myers et al., 1996), it may be speculated that increased prostacyclin formation reduced IP receptor sensitivity (Table 1).

Diabetes induced marked alterations in aortic vascular responses in male and, to a greater extent, in female rats even 1 week after streptozotocin injection. In both genders, diabetes increased contractile responses to both noradrenaline and L-NAME. Such increased contractile responses to noradrenaline (Öztürk et al., 1996) may be due to decreased endothelial prostacyclin production related to hyperglycaemia (Fujii et al., 1986; Ono et al., 1988). In contrast, the increase in aortic contractility to L-NAME reflects an increase in the basal release of NO. This may represent either a reactive response to the enhanced oxidant burden (Giugliano et al., 1996; Rösen et al., 1996) or a compensatory mechanism to the increased reactivity to noradrenaline observed shortly after diabetes onset. In addition, diabetes was associated with attenuated response to acetylcholine and with enhanced response to iloprost in tissues from female rats. Impaired acetylcholine relaxation in vascular tissues from diabetic rats (Feletou et al., 1994) and humans (Johnstone et al., 1993) may represent a hallmark of early endothelial dysfunction, as indicated by attenuated agonist-stimulated release of NO. Tissues from female rats may be more sensitive to this loss of function because of their enhanced agonist-stimulated release of NO (Hayashi et al., 1992). In contrast, concentration–response curves to iloprost were comparable in the aorta of non-diabetic and diabetic males, as previously reported (Bouchard et al., 1999), but were significantly shifted to the left in diabetic females. The clinical significance of enhanced iloprost sensitivity in these animals is unclear and warrants further investigation. Interestingly, the curve obtained in diabetic females was comparable to that of diabetic males (Fig. 5A and B), indicating that 1-week diabetes blunted the effects of steroid hormones on the vascular responsiveness to iloprost in female aorta. Overall, our results indicate that early diabetic vascular damage is more pronounced in female than in male rats. This is in

keeping with epidemiological observation showing a poorer outcome for cardiovascular disease in diabetic women than men (Sowers, 1998; Gaba et al., 1999).

Enhanced production of free radicals and reactive oxygen species lead progressively to endothelial dysfunction and impaired vascular function in diabetic animals (Pieper et al., 1997). Our results show that treatment with Leucoselect and, to a greater extent, lercanidipine prevents, at least in part, early diabetic vascular alterations. Leucoselect, a standardised grape seed extract containing polyphenols, was reported to increase serum total antioxidant activity in animals and humans (Ursini et al., 1999; Nuttall et al., 1998). This drug was chosen because it consists of a standardised mixture of polyphenols, which may provide synergistic effects with respect to those of single antioxidants. To the best of our knowledge, this is the first report on the effect of Leucoselect on vascular responsiveness ex vivo. In fact, this extract completely recovered the relaxant response to acetylcholine (Fig. 4B), whereas iloprost-mediated responses, which were so drastically altered in tissues from female diabetic rats, were only partially recovered (Fig. 5B). Decreased noradrenaline contractility was also observed in Leucoselect-treated diabetic rats. Unexpectedly, Leucoselect treatment impaired superoxide dismutase-induced aortic relaxation in tissues from female diabetic rats (Fig. 3B), which in turn was not altered compared with non-diabetic animals. Remarkably, superoxide dismutase-induced relaxation was impaired in preparations from lercanidipine-treated diabetic female rats as well (Fig. 3B), thereby suggesting a link between antioxidant treatment and exogenous superoxide dismutase activity. Overall, our experiments suggest that biologically active polyphenols exert vascular protective effects in settings of marked redox imbalance, such as that arising in the early steps of diabetes development.

At variance with Leucoselect, lercanidipine is endowed not only with antioxidant properties but also with further protective effects on the vascular wall. Consequently, this broader activity spectrum of lercanidipine led to more marked effects on aortic function than Leucoselect. Indeed, when diabetic animals were treated with lercanidipine for 1 week, most diabetes-related abnormalities were reversed. In particular, noradrenaline contractility was reduced by lercanidipine treatment in preparations from female and in those from male rats. This may well be associated with the established calcium-antagonist effect of the drug (Guarnieri et al., 1996). However, most effects of lercanidipine treatment were detectable in females only. The drug reversed fully the altered response to acetylcholine and almost completely that to iloprost. As mentioned above, relaxation to superoxide dismutase was significantly impaired in aortic tissues from lercanidipine-treated compared with that in untreated female diabetic rats (Fig. 3B). It is conceivable that lercanidipine increased reactivity of the aortic smooth muscle to oxygen-derived free radicals secondary to either alterations in calcium influx (Wang et al., 1999) or up-regulation of endogenous superoxide dismutase (Yang et al., 2000). Overall, these results do not allow dissection of the relative contribution of antioxidant vs. other mechanisms to the protective effects of lercanidipine on aortic function. Further experiments are required to address this issue.

In conclusion, gender differences apply in aortic responses to different agonists in both non-diabetic and short-term diabetic animals. However, gender differences are detectable also in response to pharmacological treatment. The vascular tissue from female rats appears to be more susceptible to oxidative damage but also more responsive to pharmacological agents with antioxidant activity. Therefore, such agents may prevent the vascular damage associated with early phases of diabetes development.

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