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The aldose reductase inhibitor sorbinil does not prevent the impairment in nitric oxide-mediated neurotransmission in anococcygeus muscle from diabetic rats

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Abstract

This study investigated whether increased polyol pathway activity could contribute to alterations in nitrergic neurotransmission in anococcygeus muscles from 8-week diabetic rats. In the presence of guanethidine (10–30 μM) and clonidine (0.01–0.05 μM), relaxations obtained to nitrergic nerve stimulation (0.5–5 Hz, 10-s train), to sodium nitroprusside (5–500 nM) and to nitric oxide (0.1–3 μM) were significantly reduced in muscles from diabetic rats compared to responses from control rats. Treatment of diabetic rats with the aldose reductase inhibitor sorbinil (42 mg/kg per day via feed for 8 weeks) did not affect impaired reactivity to nitrergic nerve stimulation, sodium nitroprusside or nitric oxide. The results suggest increased polyol pathway activity does not contribute to the alterations in nitrergic neurotransmission in anococcygeus muscles from diabetic rats.

Keywords: Aldose reductase; Anococcygeus muscle, rat; Diabetes; Nitrergic neurotransmission; Nitric oxide (NO); Sorbinil

1. Introduction

Neuropathy of the autonomic nervous system associated with diabetes mellitus can contribute to cardiovascular, gastrointestinal and genitourinary dysfunction (Hosking et al., 1978). Studies using animal models of diabetes have demonstrated morphological, histological and functional changes in autonomic neurotransmission involving noradrenergic, cholinergic, serotonergic, peptidergic and purinergic nerves (Lincoln et al., 1984; Nowak et al., 1986; Belai et al., 1988, 1991). We have recently reported that the functioning of nitric oxide-mediated (or nitrergic) neurotransmission in the anococcygeus smooth muscle from streptozotocin (STZ)-treated rats is impaired (Way and Reid, 1994a,b,1995). Relaxant responses to nitrergic nerve stimulation, sodium nitroprusside and nitric oxide were impaired, whereas relaxations to papaverine, which does not act through nitric oxide, were not altered by diabetes (Way and Reid, 1994a). Furthermore, this alteration appears to be partly mediated by a defect in the ability of cyclic guanosine 3′,5′-monophosphate (GMP) to relax the smooth muscle, as our previous findings demonstrated reduced relaxations to 8-bromo-cyclic GMP in tissues from diabetic rats (Way and Reid, 1994b). Insulin treatment of diabetic rats was shown to prevent the impairment (Way and Reid, 1995). However, treatment of rats with aminoguanidine, to inhibit advanced glycation end-product formation, did not prevent the diabetes-induced alterations, suggesting formation of these products is not involved (Way and Reid, 1994b).

In the present study, we have explored the possibility that another biochemical mechanism, the polyol pathway, might be associated with impaired nitrergic neurotransmission. Excessive flux of glucose through the polyol pathway as a consequence of hyperglycaemia leads to the intracellular accumulation of sorbitol and fructose via the enzymes aldose reductase and sorbitol dehydrogenase respectively (Gabbay, 1973). Elevated tissue levels of sorbitol and fructose have been detected in a variety of tissues from both animal models and patients with diabetes, including lens (Van Heyningen, 1959; Tilton et al., 1995), glomeruli (Beyer-Mears et al., 1984), red blood cells

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(Malone et al., 1984), peripheral nerve (Gabbay et al., 1966; Ward et al., 1972; Hale et al., 1987; Cameron and Cotter, 1992) and spinal cord (Gabbay et al., 1966; Ward et al., 1972). The polyol pathway has thus been implicated in the pathogenesis of diabetic complications including cataracts, retinopathy, nephropathy and neuropathy (Gabbay, 1973; Beyer-Mears et al., 1984).

Over the past two decades, a number of aldose reductase inhibitors have been developed and used to demonstrate the involvement of the polyol pathway in diabetes-induced nerve and vascular changes. Inhibition of aldose reductase activity can correct increased sorbitol levels and decreased axonal transport in diabetic rat sciatic nerve (Peterson et al., 1979; Yue et al., 1982; Tomlinson et al., 1984), can improve abnormalities in nerve fibre ultrastructure and morphology (Cameron et al., 1986; Schmidt et al., 1989; Yagihashi et al., 1990), and can prevent or reverse impaired motor nerve conduction velocity in both animals and humans with diabetes (Yue et al., 1982; Judzewitsch et al., 1983; Kikkawa et al., 1984; Tomlinson et al., 1984). In addition, alterations in both vascular and nonvascular smooth muscle functioning in tissues from diabetic animals can be prevented by aldose reductase inhibition (Luheshi and Zar, 1990; Cameron and Cotter, 1992; Tesfamariam et al., 1993). In the present study the reversible noncompetitive aldose reductase inhibitor sorbinil has been used to investigate whether increased polyol pathway activity could contribute to the impaired nitrergic neurotransmission observed in anococcygeus muscles from 8-week diabetic rats. This smooth muscle preparation was originally chosen for use in our studies as its nitrergic innervation has been well characterised (Li and Rand, 1989; Rand, 1992).

2. Materials and methods

2.1. Induction of diabetes with STZ

Diabetes was induced in male Sprague-Dawley rats (200–300 g) via a single tail-vein injection of STZ (65 mg/kg) dissolved in ice-cold citrate saline (20 mM, pH 4.5) immediately before use; age-matched control rats were treated with citrate saline vehicle. For the following 8-week period all animals received a diet of chow (GR2 +; Clarke King, Australia) and water ad libitum; however, STZ-treated animals received drinking water containing 2% sucrose for a 48-h period after injection to help reduce the severity of the hypoglycaemia which follows STZ treatment. Body weights of all animals were measured at intervals of 1–3 days, and STZ-treated rats were monitored for glucosuria (Tes-Tape urine sugar-analysis paper; Eli Lilly, Australia), polydipsia and polyuria. Blood samples were collected from rats after decapitation for measurement of blood glucose levels (Ames Glucometer 3; Bayer Diagnostics, Australia).

2.2. Sorbinil treatment of diabetic rats

A separate group of STZ-treated rats was administered a diet enriched with the aldose reductase inhibitor sorbinil (CP-45,634; Pfizer) for the 8-week duration of diabetes. Food was prepared according to a slight modification of the method described by Yorek et al. (1993). Sorbinil was added to ground rat chow containing 1% gum xanthan as a binding agent. After thorough mixing, the chow was repelleted by the addition of distilled water and drying in an oven at 37–42°C for 48 h. Rats received sorbinil-enriched chow ad libitum immediately after injection with STZ; consumption was monitored at intervals of 1–3 days and the average sorbinil intake was calculated to be 42 mg/kg body weight per day.

2.3. Preparation of rat anococcygeus muscles

Eight weeks after injection with vehicle or STZ, rats were killed by stunning with a blow to the head and decapitation. Anococcygeus muscles were isolated as described previously (Way and Reid, 1994a,b). In brief, following an abdominal midline incision and clearing of the genitourinary organs, the pelvis was cut and the underlying colon exposed. The colon was cut at the pelvic brim and pulled forward to reveal two anococcygeus muscles surrounded by connective tissue. The rat anococcygeus muscle consists of parallel bundles of smooth muscle fibres, and is innervated by adrenergic nerve fibres, inhibitory non-adrenergic, non-cholinergic nerves, and a small number of cholinergic fibres (Gillespie, 1980). A maximal length of each muscle was removed and mounted under 1-g tension in a 5-ml organ bath for isometric recording using a Grass FTO3C force-displacement transducer connected to a Rikadenki potentiometric recorder. Muscles were equilibrated for 20–40 min in physiological salt solution (PSS) of the following composition (mM; pH 7.4): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.03, $MgSO_4$ 0.45, D-(+)-glucose 11.1, Na_2EDTA 0.067 and ascorbic acid 0.14. The PSS was gassed constantly with 95% O_2/5% CO_2 and maintained at 37°C. All drugs were added directly into the organ bath and muscles were field stimulated via two platinum wire electrodes located on each side of the muscle. Field stimulation was delivered from a Grass S88 stimulator using square wave pulses of 0.8 ms duration and supramaximal voltage (17 V/cm).

2.4. Experimental protocols

Relaxant responses were obtained in the presence of guanethidine (10–30 μM) and clonidine (0.01–0.05 μM) to block noradrenergically mediated contractions; concentrations of guanethidine and clonidine were chosen in order to achieve a precontraction level of approximately 8–9 g tension. Clonidine was used in addition to guanethidine to raise tissue tone, as a more stable precontraction is

obtained than with guanethidine alone and the incidence of spontaneous relaxant activity in the muscles is reduced.

Relaxations were obtained to increasing frequencies of field stimulation (0.5–5 Hz, 10-s train) at 3-min intervals, to sodium nitroprusside (5–500 nM) or to nitric oxide (0.1–3 μM), each delivered in random order. Contractile responses were obtained using a separate set of tissues and in the absence of guanethidine or clonidine. Contractions were obtained to field stimulation (0.5–10 Hz, 10-s train) delivered in random order at 2-min intervals, and to cumulative additions of noradrenaline (0.03–30 μM).

2.5. Drugs and drug solutions

Sorbinil was obtained as a gift from Pfizer (Central Research Division, Eastern Point Road, Groton, CT, USA). Guanethidine sulphate, (−)-noradrenaline bitartrate, sodium nitroprusside and streptozotocin (STZ) were all purchased from Sigma (St. Louis, MO, USA). Clonidine hydrochloride was obtained from Boehringer Ingelheim (Artarmon, NSW, Australia). A saturated solution of nitric oxide (2 mM) was prepared daily by bubbling nitric oxide gas (Commonwealth Industrial Gases; Melbourne, Victoria, Australia) for 20 min into distilled water which was initially deoxygenated by gassing with argon for 1 h, as previously described (Way and Reid, 1994a,b).

2.6. Statistical analyses

Results are given as means ± S.E.M. for n number of experiments. Statistically significant differences ($P < 0.05$) between means were determined using one- or two-way analysis of variance (ANOVA) followed by planned comparisons where appropriate, with the aid of the computer package Complete Statistical Systems (CSS; Statsoft, Tulsa, OK, USA). Both EC_{50} (concentration required to produce 50% of the maximal response) and E_{max} (maximal contractile response) values for the noradrenaline concentration-response curves were determined by nonlinear regression analysis using the computer package Regression and Empirical Analytical Procedures (REAP; Gamma Research Systems, Melbourne, Victoria, Australia).

3. Results

3.1. Assessment of diabetes induction

Eight weeks after injection with STZ, blood glucose levels in rats were significantly elevated compared to levels in rats injected with vehicle ($P < 0.05$, one-way ANOVA; Table 1). Final body weights of 8-week STZ-treated rats were significantly less than weights of the vehicle-treated group ($P < 0.05$, one-way ANOVA; Table 1), but initial body weights for the two groups did not differ. Diabetes induction was further confirmed in STZ-treated animals by the appearance of glucosuria, polydipsia and polyuria.

In rats injected with STZ and treated with sorbinil, blood glucose levels remained significantly elevated compared to levels in vehicle-treated rats ($P < 0.05$, one-way ANOVA; Table 1). Final body weights were still significantly less than weights of vehicle-treated rats at 8 weeks ($P < 0.05$, one-way ANOVA; Table 1), but did not significantly differ from final body weights of STZ-treated rats ($P > 0.05$, one-way ANOVA; Table 1). In addition, the growth rates (expressed as final body weight as a percentage of initial body weight) of STZ-treated and STZ + sorbinil-treated rats were not significantly different ($P > 0.05$, one-way ANOVA), but were approximately 2-fold less than the growth rate of the vehicle-treated group ($P < 0.05$, one-way ANOVA).

Measurements of tissue weights revealed that anococcygeus muscles from vehicle-treated rats (12.9 ± 1.1 mg, $n = 16$) weighed significantly more ($P < 0.05$, one-way ANOVA) than those obtained from STZ-treated rats (6.5 ± 0.5 mg, $n = 12$) and from STZ-treated rats after treatment with sorbinil (7.8 ± 0.8 mg, $n = 11$); tissue weights from the latter two groups did not significantly differ ($P > 0.05$, one-way ANOVA).

3.2. Relaxant responses to field stimulation, sodium nitroprusside and nitric oxide

Levels of precontraction obtained to guanethidine (10–30 μM) and clonidine (0.01–0.05 μM) in muscles from

Table 1
Blood glucose levels and body weights of rats 8 weeks after treatment with vehicle (20 mM citrate saline, i.v.), streptozotocin (STZ; 65 mg/kg i.v.) or STZ and sorbinil (42 mg/kg per day via feed)

	Treatment group		
	Vehicle	STZ	STZ and sorbinil
Blood glucose (mM)	6.0 ± 0.2	22.0 ± 0.5 [a]	24.1 ± 0.9 [a]
Initial body weight (g)	262.8 ± 17.1	246.6 ± 12.6	286.2 ± 4.2
Final body weight (g)	548.0 ± 15.0	266.9 ± 12.4 [a]	272.5 ± 18.8 [a]
Final body weight (% initial weight)	214.0 ± 13.2	111.5 ± 9.7 [a]	95.1 ± 6.1 [a]

Values are means ± S.E.M. for 6–10 animals. [a] Significant difference from the vehicle-treated group ($P < 0.05$, one-way ANOVA).

control rats (9.1 ± 0.4 g, $n = 8$) did not significantly differ ($P > 0.05$, one-way ANOVA) from those levels obtained in muscles from diabetic rats (8.1 ± 0.5 g, $n = 6$). Precontraction levels achieved in tissues from sorbinil-treated diabetic rats (7.6 ± 0.4 g, $n = 5$) did not differ from levels obtained in tissues from diabetic rats ($P > 0.05$, one-way ANOVA), although they were significantly less ($P < 0.05$, one-way ANOVA) than those from the control group.

Relaxant responses to 0.5, 1, 2 and 5 Hz field stimulation (10-s train) obtained in muscles from 8-week diabetic rats (0.6 ± 0.1 g, 1.8 ± 0.2 g, 4.4 ± 0.3 g, 6.4 ± 0.5 g, $n = 5$) were significantly less ($P < 0.05$, two-way ANOVA) than those obtained in muscles from control rats (2.8 ± 0.9 g, 4.6 ± 0.8 g, 6.8 ± 0.5 g, 8.3 ± 0.4 g, $n = 6$). When data were expressed as a percentage of the initial response obtained at 5 Hz in each experiment, to assess relative differences in response size at the lower stimulation frequencies, relaxations obtained from the diabetic group remained significantly less than responses from the control group ($P < 0.05$, two-way ANOVA; Fig. 1A). Relaxations obtained to field stimulation from diabetic rats treated with sorbinil were not significantly different compared to responses from the untreated diabetic group ($P > 0.05$, two-way ANOVA; Fig. 1A), but were significantly less than responses obtained from control rats ($P < 0.05$, two-way ANOVA; Fig. 1A). To account for differences in the level of tissue tone generated between the treatment groups, relaxations were also expressed as a percentage of the precontracted tone (Fig. 1B). When expressed in this manner, relaxations obtained from the control group were again significantly greater ($P < 0.05$, two-way ANOVA; Fig. 1B) than those from both the diabetic and sorbinil-treated diabetic groups; responses from the latter two groups did not significantly differ ($P > 0.05$, two-way ANOVA; Fig. 1B).

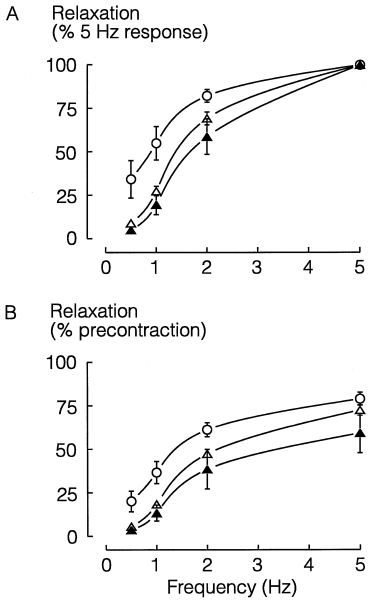

Fig. 1. Relaxant responses to field stimulation (0.5–5 Hz, 10-s train) in anococcygeus muscles from 8-week control (○), diabetic (△) and sorbinil-treated diabetic (▲) rats. Relaxations were obtained in the presence of guanethidine (10–30 μM) and clonidine (0.01–0.05 μM). Responses are means ± S.E.M. from 5–6 experiments, expressed as (A) percentages of the 5 Hz response obtained, or (B) percentages of the level of precontracted tone.

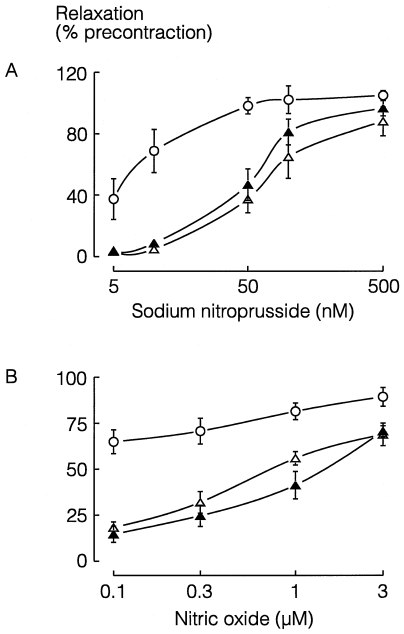

Fig. 2. Relaxant responses to sodium nitroprusside (A; 5–500 nM) and to nitric oxide (B; 0.1–3 μM) in anococcygeus muscles from 8-week control (○), diabetic (△) and sorbinil-treated diabetic (▲) rats. Responses are means ± S.E.M. from 5–6 experiments expressed as percentages of the level of precontracted tone. Tissues were precontracted with guanethidine (10–30 μM) and clonidine (0.01–0.05 μM).

Responses obtained to exogenously added relaxing agents are shown expressed as a percentage of the precontracted tone (Fig. 2). Relaxations to sodium nitroprusside (5–500 nM; Fig. 2A) and to nitric oxide (0.1–3 μM; Fig. 2B) in tissues from 8-week diabetic rats were significantly reduced compared to the corresponding control group ($P < 0.05$, two-way ANOVA). Relaxant responses obtained to both sodium nitroprusside and nitric oxide in muscles from sorbinil-treated diabetic rats did not significantly differ ($P > 0.05$, two-way ANOVA; Fig. 2) from those obtained from untreated diabetic rats.

3.3. Contractile responses to field stimulation and to noradrenaline

In the absence of guanethidine and clonidine, and when expressed as increases in g tension, contractile responses to increasing frequencies of field stimulation (0.5–10 Hz, 10-s train) and to cumulative additions of noradrenaline (0.03–30 μM), were significantly greater ($P < 0.05$, two-way ANOVA; Fig. 3A, Fig. 4A) in tissues from control rats than those from diabetic rats, or from sorbinil-treated diabetic rats; responses from both untreated and treated

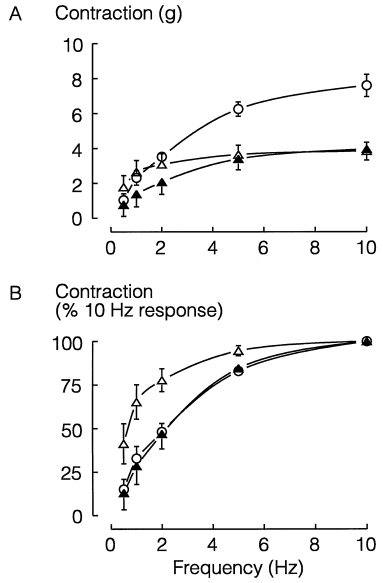

Fig. 3. Contractile responses to field stimulation (0.5–10 Hz, 10-s train) in anococcygeus muscles from 8-week control (○), diabetic (△) and sorbinil-treated diabetic (▲) rats. Responses are means ± S.E.M. from 5–8 experiments, expressed as (A) absolute increases in tension, or (B) percentages of the 10 Hz response obtained.

diabetic rats were not significantly different from each other ($P > 0.05$, two-way ANOVA; Fig. 3A, Fig. 4A). However, when contractions were expressed as a percentage of the response obtained at 10 Hz, to assess the relative differences in tissue responsiveness at the lower stimulation frequencies between the groups, contractile responses to field stimulation were significantly greater in

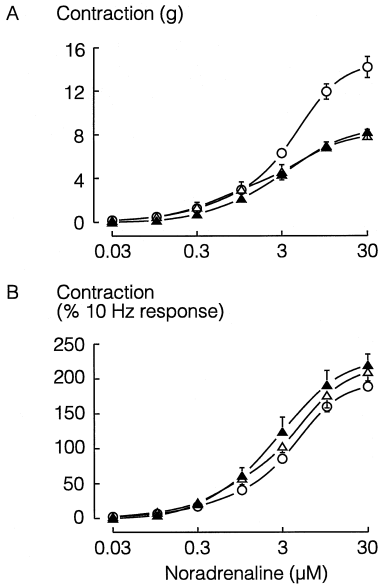

Fig. 4. Contractile responses to cumulative additions of noradrenaline (0.3–30 μM) in anococcygeus muscles from 8-week control (○), diabetic (△) and sorbinil-treated diabetic (▲) rats. Responses are means ± S.E.M. from 3–8 experiments, expressed as (A) absolute increases in tension, or (B) percentages of the 10 Hz response obtained.

Table 2
EC_{50} values and maximal contractile responses (E_{max}) for noradrenaline obtained in anococcygeus muscles from 8-week control, diabetic and sorbinil-treated diabetic rats

	Treatment group		
	Control	Diabetic	Sorbinil-treated diabetic
EC_{50} [a]	−5.4 ± 0.1	−5.4 ± 0.1	−5.5 ± 0.1
E_{max} [b]	203.7 ± 4.1	230.2 ± 13.5 [c]	234.5 ± 11.3 [c]

Values are means ± S.E.M. for 3–8 animals. [a] EC_{50} values are expressed as $\log_{10}$ of the molar concentration required to produce 50% of the maximum response. [b] E_{max} values are calculated as a percentage of the 10 Hz response to field stimulation obtained. [c] Significant difference from the control group ($P < 0.05$, one-way ANOVA).

tissues from diabetic rats ($P < 0.05$, two-way ANOVA; Fig. 3B) than those from both control rats and sorbinil-treated diabetic rats. Contractions obtained for the sorbinil-treated group were not significantly different from responses obtained for the control group ($P > 0.05$, two-way ANOVA; Fig. 3B).

Concentration-response curves obtained to cumulative additions of noradrenaline did not significantly differ between tissues obtained from any of the treatment groups, when values were expressed as percentages of the 10 Hz response ($P > 0.05$, two-way ANOVA; Fig. 4B). EC_{50} values determined for noradrenaline were not significantly different between tissues obtained from any of the treatment groups ($P > 0.05$, one-way ANOVA; Table 2). Estimated maximal responses (E_{max}) to noradrenaline, calculated as a percentage of the 10 Hz response to field stimulation, did not significantly differ between the diabetic and sorbinil-treated diabetic groups ($P > 0.05$, one-way ANOVA; Table 2); however, values for both the untreated and treated diabetic groups were significantly greater than that for the control group ($P < 0.05$, one-way ANOVA; Table 2).

4. Discussion

Consistent with our previous reports (Way and Reid, 1994a,b,1995), relaxant responses obtained to stimulation of nitrergic nerves, to the nitric oxide-donor sodium nitroprusside and to nitric oxide, were reduced in anococcygeus muscles from 8-week STZ-treated rats. In the present study we have investigated whether increased glucose flux through the aldose reductase pathway could contribute to the impairment in nitrergic neurotransmission. The aldose reductase inhibitor sorbinil was administered to diabetic rats via the feed at an oral dose of 42 mg/kg body weight per day; this treatment did not alter body weights or blood glucose levels. Oral administration of sorbinil to experimental animals has been extensively used to inhibit aldose reductase in diabetic studies. Although we did not assess the effectiveness of sorbinil administration to reduce sor-

bitol levels in the present study, oral doses between 0.25 and 50 mg/kg per day have been previously demonstrated to prevent sorbitol accumulation in various tissues from diabetic rats (Peterson et al., 1979; Yue et al., 1982; Malone et al., 1984; Schmidt et al., 1989). In the present study sorbinil treatment of diabetic rats for 8 weeks did not reduce the impairment of nitrergic neurotransmission in anococcygeus muscles, suggesting that the changes are not related to increased activity of the polyol pathway induced by elevated glucose levels.

As described in the Introduction of this paper, many studies have reported the beneficial effects of aldose reductase inhibitors in preventing or reducing diabetes-induced defects. However, consistent with the findings in the present study, some reports also show a lack of effect of a variety of aldose reductase inhibitors, including sorbinil: tolrestat did not prevent decreased nerve blood flow and motor nerve conduction velocity in sciatic nerve from 8-week diabetic rats (Calcutt et al., 1994); ponalrestat had no effect on increased vascular reactivity to noradrenaline, or impaired relaxations to acetylcholine in the isolated perfused mesentery of diabetic rats (Taylor et al., 1994), and did not improve chronic neuropathic symptoms in diabetic patients (Florkowski et al., 1991); and sorbinil did not prevent retinopathy in alloxan diabetic dogs (Engerman and Kern, 1993), or changes in albumin permeation or urinary excretion in diabetic rats (Tilton et al., 1991), and did not improve abnormal nerve function in diabetic rats (Gillon et al., 1983) or patients with diabetes (Lewin et al., 1984; Martyn et al., 1987).

The ability of nitric oxide released from nitrergic nerves to modulate noradrenergically mediated contractile responses is well established in the rat anococcygeus muscle (Li and Rand, 1989; Way and Reid, 1994a). In the present study, and consistent with our previous results (Way and Reid, 1994a), stimulation-induced contractions were significantly enhanced in tissues from diabetic rats compared to control responses, when contractions were expressed as a percentage of the 10 Hz response to assess for relative differences in response size (see Li and Rand, 1989; Way and Reid, 1994a). We have previously suggested that the enhancement results from an impairment in the ability of nitric oxide to modulate noradrenergically mediated responses in muscles from diabetic rats; in support of this suggestion enhancement of stimulation-induced contractile responses by the nitric oxide synthase inhibitor N^G-nitro-L-arginine was less in muscles from diabetic rats than from control rats (Way and Reid, 1994a). However, results in the present study demonstrate that although sensitivity to noradrenaline did not differ between the treatment groups, maximum tension generated by noradrenaline from both the untreated and treated diabetic groups was slightly greater than for the control group. It is possible that this may also contribute to the relative enhancement of noradrenergically mediated contractions in muscles from diabetic rats.

The effect of sorbinil treatment on stimulation-induced contractions in muscles from diabetic rats was unexpected. Sorbinil treatment of diabetic rats reduced stimulation-induced contractile responses to the levels obtained in tissues from control rats, but did not affect responsiveness of tissues to exogenously added noradrenaline, when data were expressed as a percentage of the 10 Hz response. These findings could suggest that noradrenaline content or release from noradrenergic nerves may be increased in anococcygeus muscles from diabetic rats as a consequence of increased polyol pathway activity. Diabetes-induced increases in tissue noradrenaline content have been previously demonstrated in rat colon (Lincoln et al., 1984; Belai et al., 1988), vagus nerve (Dhital et al., 1986) and heart (Ganguly et al., 1986). However, if both a decrease in the modulatory action of nitric oxide and an increase in noradrenaline release contribute to enhanced stimulation-induced contractions in diabetic tissues, sorbinil treatment should only partially rather than fully reverse the alterations, given that sorbinil did not prevent the diabetes-induced impairment in nitrergic neurotransmission. Moreover, sorbinil may have a direct effect to inhibit the stimulation-induced release of noradrenaline in this preparation; however, this cannot be determined from the present study because sorbinil was not simultaneously administered to control rats.

In contrast, when data were expressed as absolute increases in g tension, contractions to field stimulation and to noradrenaline were significantly less in tissues from 8-week diabetic rats. More importantly, sorbinil treatment did not prevent the impaired contractions to nerve stimulation and noradrenaline in tissues from diabetic rats, suggesting that any diabetes-induced changes in noradrenergic neurotransmission are not related to increased activity of the polyol pathway. Therefore, the effect of sorbinil on noradrenergically mediated contractions in diabetic tissues appears to depend on the method of data expression. It is concluded that a role for increased polyol pathway activity is unlikely, and the contrasting effects of sorbinil may be an artefact resulting from the alternate method of data expression.

In conclusion, the impairment in nitrergic neurotransmission in the anococcygeus muscle from 8-week diabetic rats does not appear to be related to an increase in the activity of the polyol pathway as sorbinil did not prevent or reduce the alterations. Therefore, the mechanisms or biochemical pathways which underlie the impaired nitrergic neurotransmission in anococcygeus muscles from diabetic rats still remain to be determined.

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