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European Journal of Pharmacology 286 (1995) 193–199

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Effects of streptozotocin-diabetes on sympathetic nerve, endothelial and smooth muscle function in the rat mesenteric arterial bed

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Received 22 June 1995; accepted 21 July 1995

Abstract

Mesenteric arterial function was assessed in constantly perfused preparations isolated from rats 12 weeks after treatment with streptozotocin (65 mg kg^{-1}, i.p.) to induce diabetes. Frequency-dependent vasoconstrictor responses to electrical field stimulation of sympathetic nerves (4–32 Hz, 0.1 ms, 90V, 30 s) were severely attenuated in preparations from streptozotocin-diabetic rats, although dose-dependent vasoconstrictions to the sympathetic cotransmitters noradrenaline and ATP, as well as to potassium chloride, were not significantly changed. Dose-dependent relaxations to the endothelium-dependent vasodilators acetylcholine and ATP were significantly impaired in preparations from streptozotocin-diabetic rats, although endothelium-independent vasodilatation to sodium nitroprusside was unimpaired. These results suggest that 12 weeks after induction of streptozotocin-diabetes in rats there is pre-junctional impairment of sympathetic neurotransmission and impaired endothelial function of the mesenteric arteries. This is in contrast to our previous findings that at 8 weeks after induction of streptozotocin-diabetes sympathetic nerve and endothelial function is normal, although sensory-motor vasodilatation is severely attenuated. It is suggested that selective changes occur in mesenteric arterial function after streptozotocin treatment depending on the duration of diabetes; sensory-motor nerves are affected first, followed by sympathetic nerves and the endothelium, while the smooth muscle is relatively resistant to change.

Keywords: Endothelium; Mesenteric arterial bed, rat; Streptozotocin diabetes; Sympathetic nerve

1. Introduction

The vascular complications which occur in diabetes mellitus typically involve malfunction of sensory, motor and autonomic nerves and endothelial cells. Much information on the pathology of diabetes has been obtained using animal models particularly the streptozotocin-treated rat, a model of insulin-dependent diabetes. However, little information is available about the relative susceptibilities of perivascular nerves, endothelium and smooth muscle to damage due to this disease. The progression of changes in the vasculature in diabetes is difficult to ascertain from a review of the literature alone because of the notorious lack of consensus between different laboratories even when using the same vascular preparations and similar times and methods of induction of diabetes.

Our own recent findings have added to the body of conflicting reports by showing that after 8 weeks of streptozotocin-diabetes vasoconstrictor responses of the rat mesenteric arterial bed to electrical field stimulation (EFS) of sympathetic nerves and to exogenous noradrenaline (NA) were unimpaired (Ralevic et al., 1993). In contrast, vasodilatation due to EFS of sensory-motor nerves was severely attenuated (Ralevic et al., 1993). While this is in line with the results of workers who have also shown that constrictor responses to NA are unchanged in diabetic rat mesenteric arteries (Fortes et al., 1983; Furman and Sneddon, 1993) it is in contrast to other studies which variously report that responses to sympathetic nerve stimulation and exogenous constrictors are attenuated (Takiguchi et al., 1988; Longhurst and Head, 1985; Andersson et al., 1992), or increased (Agrawal and McNeil, 1987; Abebe and MacLeod, 1990; Abebe et

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SSDI 0014-2999(95)00451-3

al., 1990; White and Carrier, 1990; Taylor et al., 1992, 1994a,b). We also found that endothelium-dependent vasodilatation of rat mesenteric resistance vessels was unimpaired at 8 weeks of steptozotocin-diabetes (Ralevic et al., 1993), again in agreement with some (Furman and Sneddon, 1993), but not other studies (Taylor et al., 1992).

The aim of the present study was to determine whether there is a relationship between the changes which occur in vascular function due to diabetes and the duration of this disease. Specifically, we aimed to investigate whether the lack of changes in function of sympathetic nerves and endothelium at 8 weeks after streptozotocin treatment (Ralevic et al., 1993) could be overcome by a longer duration (12 weeks) of streptozotocin-diabetes.

2. Materials and methods

2.1. Animals

Diabetes was induced in adult male Wistar rats (weighing 400–450 g) by a single intraperitoneal injection (65 mg kg^{-1} body weight) of buffered streptozotocin (Belai et al., 1988; Ralevic et al., 1993). Controls were untreated age-matched rats of the same initial weight range. The onset of diabetes was established by the presence of rapid weight loss, polyuria and glycosuria. Both groups were maintained under the same conditions, for 12 weeks, supplied with food and water ad libitum until death. Previous studies in this laboratory showing no changes in a subgroup of streptozotocin-injected rats which failed to develop diabetes at any time during the 8 week period after streptozotocin injection are consistent with this model being one of streptozotocin-induced diabetes, rather than of the effects of streptozotocin per se. Blood samples were taken from the posterior vena cava for blood glucose analysis under ether asphyxiation.

2.2. Mesenteric arterial bed preparation

Mesenteric arterial beds were isolated and set up for perfusion essentially as described previously (Ralevic and Burnstock, 1988). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed, the gut dissected away and the preparation mounted on a stainless steel grid (7 cm × 5 cm) in a humid chamber. The preparation was perfused at a constant flow rate of 5 ml min^{-1} using a peristaltic pump (Cole Parmer Instruments). Perfusion was with Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2 – 5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23, Gould) on a side arm of the perfusion cannula, and recorded on a polygraph (model 79D, Grass). The preparation was allowed to equilibrate for 30 min prior to experimentation.

Stimulation of perivascular nerves was achieved by passing a current between the cannulation needle and the wire grid on which the preparation rested. Sympathetic nerves were activated at basal tone by electrical field stimulation (90 V, 1 ms, 4–32 Hz for 30 s). The resulting vasoconstrictions could be abolished by guanethidine treatment. Vasoconstrictor responses to bolus injections of doses NA and ATP were also tested at basal tone with doses administered at 3 min intervals or after tone had returned to baseline. To examine vasodilator responses to ACh, ATP and SNP the tone of the preparations was raised by the addition of methoxamine to the perfusate to a final concentration of 3–300 μM. Intervals between doses at raised tone were determined by the time it took for tone return to its preconstricted level. Constrictor responses to potassium chloride (KCl, 0.15 mmol), at basal tone (following washout of methoxamine), were established at the end of each experiment as a measure of the contractile potential of the vascular smooth muscle. Drugs were administered as 50 μl bolus injections via an injection port proximal to the tissue.

2.3. Drugs

Acetylcholine chloride, adenosine 5′-triphosphate (disodium salt) sodium nitroprusside, noradrenaline bitartrate and methoxamine hydrochloride were obtained from Sigma, Poole, England. All drugs were

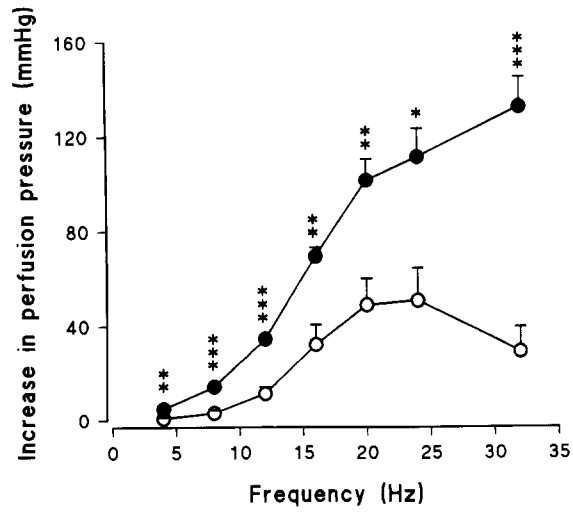

Fig. 1. Frequency-response curves showing that streptozotocin-diabetes impairs vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to electrical field stimulation of sympathetic nerves (4–32 Hz, supramaximal voltage, 1 ms for 30 s): (●) control ($n = 6$); (○) streptozotocin-diabetic ($n = 4$).

made up in distilled water, except for NA, which was made up as a 10 mM stock solution in 0.1 mM ascorbic acid. Streptozotocin was donated by the Division of Cancer Treatment, National Institutes of Health, Bethesda, MD.

2.4. Data analysis

All results were expressed as the means ± S.E. Data analysis was done by Students' *t*-test. $P < 0.05$ was taken as significant.

3. Results

3.1. Diabetic model

The untreated controls gained weight during the 12 week period, reaching a final body weight of 669.4 ± 32.2 g ($n = 6$). The streptozotocin-treated rats lost weight over this period; final body weight was 403.7 ± 15.2 g ($n = 4$). All diabetic rats used in the present study were severely hyperglycaemic with blood glucose levels of 49.6 ± 1.9 mmol l^{-1} ($n = 4$). Blood glucose levels of control rats was 14.3 ± 0.8 mmol l^{-1} ($n = 6$). Distention of the large and small intestines and the presence of pale watery stools were observed at the time of death as previously described (Belai and Burnstock, 1990).

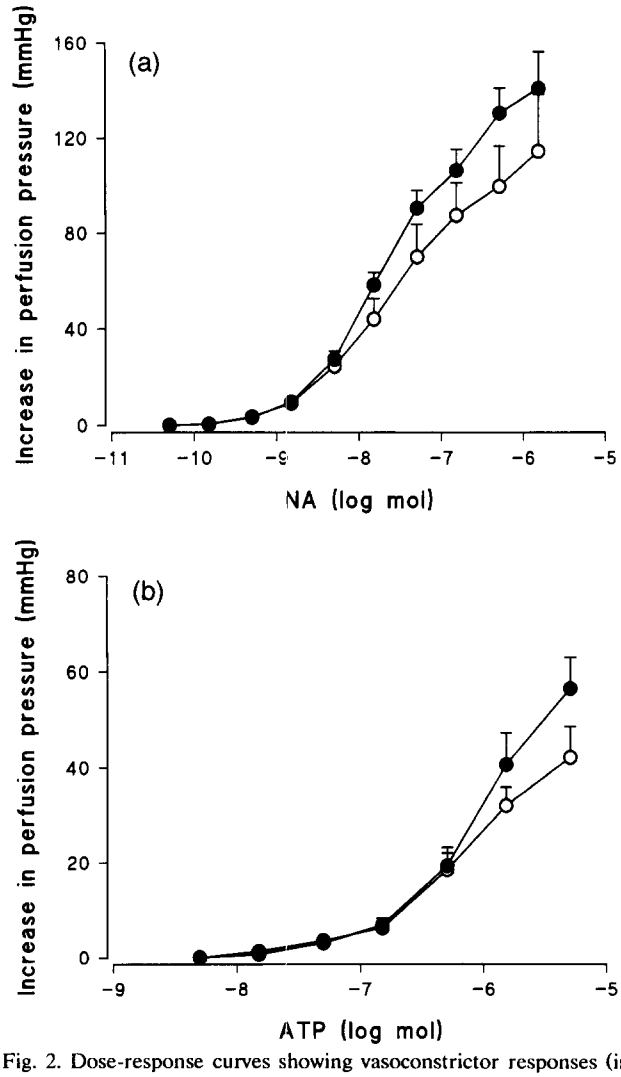

Fig. 2. Dose-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to (a) exogenous noradrenaline (NA) and (b) ATP: (•) control ($n = 6$); (○) streptozotocin-diabetic ($n = 4$).

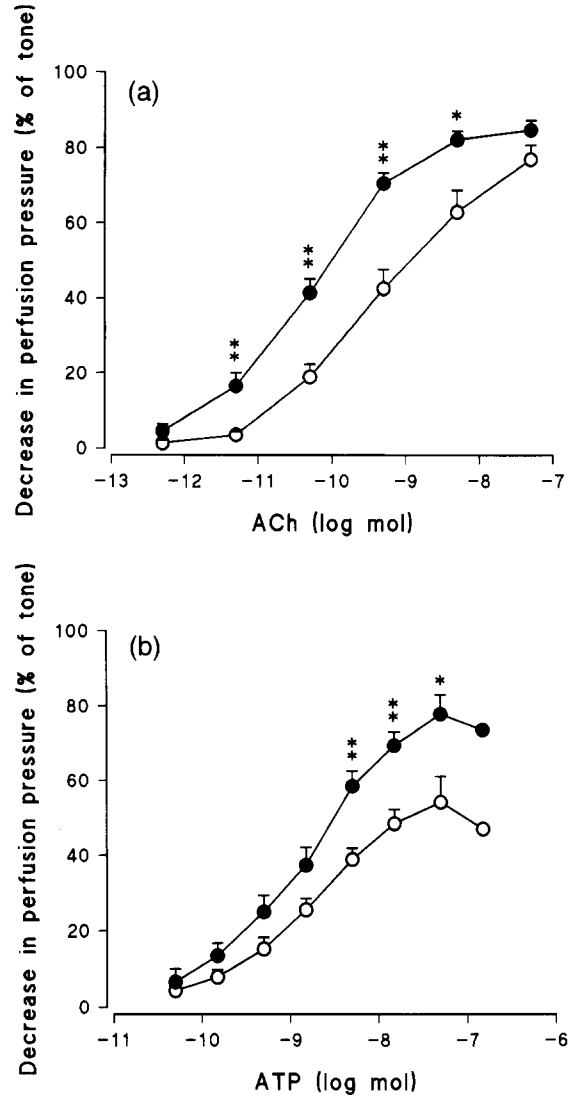

Fig. 3. Dose-response curves showing impaired endothelium-dependent relaxations (decrease in perfusion pressure, mmHg) to: (a) acetylcholine (ACh) and (b) ATP in mesenteric arterial beds from streptozotocin-diabetic rats. (•) Control ($n = 6$); (○) streptozotocin-diabetic ($n = 4$).

3.2. Basal tone

Diabetic rats had significantly lower basal perfusion pressures than the control group ($P < 0.01$). Basal perfusion pressures were: 33.8 ± 3.7 ($n = 6$) and 25.3 ± 1.0

($n = 4$) mmHg in mesenteric beds from control and streptozotocin-diabetic rats respectively.

3.3. Vasoconstrictor responses to sympathetic nerve stimulation

EFS of sympathetic nerves at basal tone elicited frequency-dependent vasoconstrictor responses of the mesenteric vascular beds. Responses of mesenteric beds from streptozotocin-diabetic rats were severely impaired compared to those of controls (Fig. 1).

3.4. Vasoconstrictor responses to noradrenaline, ATP and KCl

Constrictor responses to NA and ATP were similar in mesenteric beds from control and streptozotocin-diabetic rats (Fig. 2). There was a trend for the highest doses of NA and ATP to be reduced in streptozotocin-diabetic preparations but this did not reach statistical significance. There was no significant difference in constrictor responses to 0.15 mmol KCl between the groups; 94.5 ± 20.2 mmHg ($n = 6$) in controls and 78.5 ± 17.5 ($n = 4$) in streptozotocin-diabetics.

3.5. Vasodilator responses to acetylcholine, ATP and sodium nitroprusside

There was no difference in the increase in tone above baseline produced by methoxamine in control (95.0 ± 11.9 mmHg, $n = 6$) and streptozotocin-diabetic (74.4 ± 13.9 mmHg, $n = 4$) rats. Vasodilator responses to ACh and ATP were attenuated across the range of doses shifting to the right the dose-response curves (Fig. 3a,b). Endothelium-independent vasodilatation to SNP was unimpaired (Fig. 4).

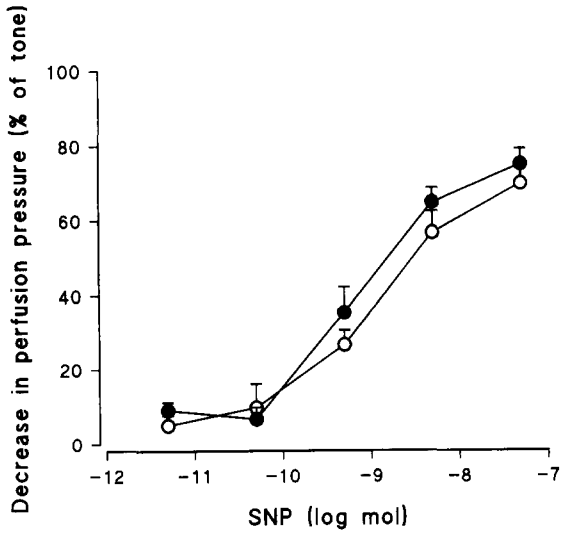

Fig. 4. Dose-response curves showing vasodilator responses of rat mesenteric arterial beds to sodium nitroprusside (SNP). Control (●, $n = 6$); streptozotocin-diabetic (○, $n = 4$).

4. Discussion

Diabetic neuropathy and impaired endothelial cell function in diabetes has been described extensively. However, little is known about the relative susceptibilities of perivascular nerves, endothelium and smooth muscle to malfunction in this disease. The isolated mesenteric arterial bed of the rat is a valuable model in which to study vascular changes in diabetes and other vascular disorders since it allows examination of the function of sympathetic and sensory-motor perivascular nerves, endothelium and vascular smooth muscle. Responses elicited by the preparation are to a large extent representative of the function of mesenteric resistance vessels, known to contribute importantly to systemic blood pressure.

The marked sympathetic neuropathy seen in the present study was due to prejunctional changes, since there was no significant difference in postjunctional constriction to the sympathetic cotransmitters NA and ATP. This is in contrast to the normal responses of sympathetic nerves seen by us in rat mesenteric arteries at 8 weeks after induction of streptozotocin-diabetes (Ralevic et al., 1993). Reports on the nature and severity of changes in the mesenteric vasculature in diabetes differ widely, reflecting the use of different methods of induction of diabetes, route of administration of the diabetogenic agent and sex and age of the rats. Thus, to make direct comparisons between these studies is difficult. Since we used identical conditions in the present study as those used previously (Ralevic et al., 1993), with the exception of increasing the duration of diabetes from 8 to 12 weeks, it was possible to draw conclusions about the effects of duration of diabetes on mesenteric function. The fact that at 8 weeks after streptozotocin treatment there was no change in responses to EFS of sympathetic nerves suggests a relative resistance of these nerves to malfunction compared to sensory-motor nerves, for which responses were significantly attenuated (Ralevic et al., 1993). The results of the present study showed that this resistance could be overcome when the duration of diabetes was increased to 12 weeks. A greater vulnerability of sensory-motor over sympathetic nerves in the rat mesenteric arterial bed has also been shown to occur after long-term senna treatment (Ralevic et al., 1990).

The cause of diabetic neuropathy, an axonal 'dying back' type of neuropathy, remains unresolved although there is a substantial body of evidence implicating metabolic dysfunction through disorders of phospho-

inositide pathways (see Greene et al., 1989). Alteration of axonal transport proteins occurs before there is pathological change but ultimately there is a decrease in nerve conduction velocities, axonal degeneration and atrophy and segmental demyelination (Brown et al., 1980; Dyck, 1982; Greene et al., 1989). Motor, sensory and autonomic nerves are all affected, however, it is interesting that peripheral sensory nerves respond rather differently to treatment compared with motor nerves, with diabetic abnormalities being relatively resistant to improvement after insulin and dietary treatment (Gregersen, 1968; Ward et al., 1971; Porte et al., 1981). Moore et al. (1980), in a comparative study of sural and tibial nerve sensory and motor conduction velocities, showed that sensory conduction velocity was slowed earlier and more frequently than motor conduction velocity. It is possible that increasing axon length produces decreased stability which would account for the greater vulnerability of sensory-motor nerves due to their long axons and the remoteness of the nerve ending from the cell body in dorsal sensory ganglia.

Lack of tone due to sympathetic neuropathy might contribute to the lower basal perfusion pressure seen in streptozotocin-diabetic preparations in the present study. On the other hand, a decreased basal perfusion pressure in experimental diabetes has been reported by us previously when there was no evidence for functional changes in sympathetic neurotransmission or smooth muscle vasoconstriction (Ralevic et al., 1993). It is possible that this may be due to an increase in vessel calibre as a consequence of diabetic hyperphagia and increase in mesenteric blood flow. Mesenteric vasodilatation in streptozotocin-diabetes has also been described in vivo (Kiff et al., 1991). The differences in tone are unlikely to have influenced the results of the present study since sympathetic and endothelial function was selectively impaired, with no changes observed in responses to NA, ATP, KCl and SNP.

Significantly impaired endothelium-dependent vasodilatation seen at 12 weeks in the present study, in contrast to the lack of change at 8 weeks (Ralevic et al., 1993), indicates that endothelial cells are also more resistant to change in diabetes than sensory-motor nerves. This was not due to a change in the ability of the smooth muscle to relax since responses to SNP were unaltered. If the degree of attenuation of responses is proportional to the degree of malfunction the present results indicate that in rat mesenteric arteries sympathetic nerves are more vulnerable to change in streptozotocin-diabetes than are endothelial cells. An early suggestion of differential impairment of components of the vasculature in diabetes was provided by Takiguchi and coworkers (1988) who showed that sympathetic neuropathy occurred at 8 weeks after induction of streptozotocin-diabetes, while endothelial damage occurred much later, at 12 weeks. In the present study, the absence of changes in postjunctional responses to NA, ATP and KCl at 12 weeks indicates a relative resistance of the vascular smooth muscle to change due to diabetes. It is possible that an even longer period of diabetes would have produced changes in the smooth muscle. A decrease in contractile responses to KCl has been shown in the rat mesenteric arterial bed (Longhurst and Head, 1985), and to endothelin, KCl and calcium chloride in the rat aorta (Fulton et al., 1991). However, the duration of streptozotocin treatment had to be balanced against the health of the animals which had started to deteriorate at 12 weeks.

Impaired endothelium-dependent relaxation was seen for both ACh and ATP, suggesting a dysfunction of endothelial cell mechanisms rather than in specific cell surface receptors to either of these agents. Impaired endothelium-dependent relaxation has been described by other workers in clinical (De Tejada et al., 1989) and experimental (Takiguchi et al., 1988; Kamata et al., 1989; Kiff et al., 1991; Mayhan, 1992; Miyata et al., 1992; Taylor et al., 1992; Abiru et al., 1993) diabetes. In rat mesenteric arteries this is likely to be due to a reduced release of endothelium-derived relaxing factor/nitric oxide (Furchgott, 1983) rather than to changes in vasoconstrictor or vasodilator products of the cyclo-oxygenase pathway since indomethacin has been shown to have no significant effect on ACh-induced relaxations in control or diabetic preparations (Taylor et al., 1992). A decrease in ACh-evoked release of cGMP in the diabetic rat aorta (Kamata et al., 1989) and mesentery (Abiru et al., 1993), taken as an indication of an impairment of the vascular endothelium, has been shown although in the latter study cAMP production was also reduced. A progressive impairment of ACh-mediated endothelium-dependent relaxation with duration of diabetes has recently been described in the rat aorta (Orie et al., 1993).

We and others have previously shown that responses to SNP are augmented following inhibition of NO synthase (Lüscher et al., 1989; Moncada et al., 1991; Jackson and Busse, 1991; Ralevic et al., 1991). It has been suggested that elevation of vascular smooth muscle cGMP by endothelial NO mediates the depression of nitrovasodilator activity (Jackson and Busse, 1991). In the present study, responses to SNP were not increased despite attenuation of endothelium-dependent relaxation. It is possible that if impaired endothelium-dependent relaxation in the present study does involve NO synthase, the decrease in production of NO in the diabetic mesenteric arteries may not have been as great as that which can be achieved by selective inhibition of NO synthase. It is also possible that impaired endothelium-dependent relaxation in the present study involves NO-independent, cGMP-independent mechanisms, one

possibility being endothelium-derived hyperpolarizing factor, which activates potassium channels in the vascular smooth muscle (Rubanyi, 1991).

In conclusion, we have identified in streptozotocin-induced diabetic rat mesenteric arteries an order of susceptibility to malfunction of: sensory-motor nerves > sympathetic nerves ≥ endothelium ≫ smooth muscle. It is suggested that this may provide a measure for assessment of the severity of diabetic vascular disorder.

Acknowledgements

This study was supported by the British Heart Foundation.

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