British Journal of Pharmacology (1995) 116, 2983-2988

Effects of chronic vitamin E deficiency on vascular function – a study of sympathetic nerves, smooth muscle and endothelium of the mesenteric arterial bed of the rat

[1]Vera Ralevic, *Peter J. Milla & Geoffrey Burnstock

Department of Anatomy and Developmental Biology and Centre for Neuroscience, University College London, Gower Street, London WC1E 6BT and *Division of Surgery, Institute of Child Health, Guildford Street, London WC1N 1EH.

1 Male rats were deprived as weanlings of dietary vitamin E for 2, 4, 6, 10 and 12 months. Mesenteric arterial beds from these rats and from age-matched controls were isolated and perfused with Krebs solution at a constant flow rate (5 ml min^{-1}). The function of perivascular sympathetic nerves, smooth muscle and endothelium was assessed.

2 At 12 months vitamin E deficient rats exhibited the characteristic symptoms of vitamin E deficiency, namely poor coat condition, muscle wasting, kyphoscoliosis and impaired gait. In the isolated mesenteric arterial bed electrical field stimulation (EFS) of perivascular nerves (4–32 Hz, 90 V, 1 ms, for 30 s) elicited frequency-dependent vasoconstrictor responses which were unaffected by vitamin E deficiency except at 12 months, at which age responses were significantly greater than those of the controls at 24 and 32 Hz ($P<0.01$).

3 Exogenous noradrenaline (NA; 0.15–500 nmol) elicited dose-dependent vasoconstriction which was similar in vitamin E-deficient and control preparations at all ages. Potassium chloride (0.15 mmol) also produced similar vasoconstrictor responses in vitamin E-deficient and control preparations at each age.

4 Tone of the preparations was raised by continuous perfusion with methoxamine (4–70 μM), producing similar increases in perfusion pressure in vitamin E-deficient and control preparations at each age. Endothelium-dependent dose-dependent vasodilatation to adenosine 5'-triphosphate was significantly impaired in mesenteric arterial beds from 12 month-old vitamin E-deficient rats compared with the controls ($P<0.05$). Relaxation to acetylcholine was not significantly different at any age.

5 Endothelium-independent vasodilatation to sodium nitroprusside was similar in vitamin E-deficient rats and age-matched controls.

6 These results suggest that long term (12 months) deprivation of dietary vitamin E may impair endothelial function in mesenteric arteries of the rat. Sympathetic perivascular nerve constrictor function was increased at 12 months. There were no functionally expressed changes in the vascular smooth muscle, which appears to be more resilient to the effects of oxidative stress in vitamin E deficiency.

Keywords: Antioxidant; endothelium; rat mesenteric arteries; sympathetic perivascular nerves; vitamin E deficiency

Introduction

Highly reactive free radical species have been implicated in damage to biological tissues formed during various biological reactions as well as in ageing and in the development of certain chronic diseases (Olson & Kobayashi, 1992; Packer, 1992; Simonoff *et al.*, 1992). Vitamin E (comprising a family of tocopherols and trienols) is possibly the most important lipid-soluble, endogenous modulator of oxidative processes. It acts as part of a complex system of biological antioxidants to quench peroxyl radicals thus interrupting the chain-propagating process of peroxyl radical formation (Burton *et al.*, 1983; Muller & Goss-Sampson, 1990; Van Acker *et al.*, 1993). In addition, it may modulate other signalling processes such as the formation of prostanoids, hydroxyeicosatetraenoic acid and sterols (Packer, 1992). The antioxidant properties of vitamin E are important in maintaining the integrity and stability of biological membranes by protecting unsaturated fatty acids of membrane phospholipids from peroxidation. Neural tissues appear to be particularly susceptible to a chronic deficiency of vitamin E and neuropathies involving peripheral and central nervous systems have been described (Harding, 1987; Muller & Goss-Sampson, 1990; Sokol, 1990).

Few studies have specifically addressed the consequences of vitamin E deficiency on vascular function. In rats, long-term dietary vitamin E deprivation was associated with impaired endothelium-dependent relaxation of the aorta (Rubino & Burnstock, 1994) and superior mesenteric artery (Hubel *et al.*, 1989). A protective effect of vitamin E against oxidative injury has been shown in human endothelial cells in culture (Kaneko *et al.*, 1991). In an electrophysiological study of the onset and severity of abnormalities associated with vitamin E deficiency Goss-Sampson and coworkers (1990) showed a delay in conduction velocity of central nerves at 8 months of deficiency and in peripheral sensory-motor nerves at 11 months of deficiency. The effects of vitamin E deficiency on perivascular nerve function are not known.

The susceptibility of neural tissue and endothelial cells to damage due to free radical attack raises important questions about their function in conditions of compromised antioxidant status such as in vitamin E deficiency. The aim of the present study was to examine the effects of long-term vitamin E deficiency on the function of components of the blood vessel wall, namely perivascular sympathetic nerves, vascular smooth muscle and endothelium, using as a model system the *in vitro* perfused mesenteric arterial bed of the rat. Mesenteric arterial beds were isolated from rats at 2, 4, 6, 10 and 12 months after dietary vitamin E deficiency and from age-matched controls to determine the onset and severity of vitamin E-associated abnormalities.

[1]Author for correspondence.

Methods

Animals and diet

Weanling (21–23 day), specific pathogen-free, male Wistar rats were obtained from Charles Rivers Limited, U.K. One group was placed on a vitamin E-deficient diet (vitamin-free casein, dextrose, stripped lard diet; Machlin/Draper-HLR no. 814, supplied by Dyets, PA, U.S.A.) as previously described (Goss-Sampson *et al.*, 1990). The vitamin E content of this diet was determined to be less than 1 ng ml^{-1} (Dyets). Another group of rats (controls) received the same diet to which α-tocopheryl acetate (vitamin E; 100 mg kg^{-1} diet) was added. Food and water were provided *ad libitum*. Rats were used at 2, 4, 6, 10 and 12 months.

Isolated mesenteric arterial bed preparation

Rats were killed by sodium pentobarbitone overdose. Mesenteric beds were isolated and set up for perfusion by a modification of the technique originally described by McGregor (1965) as described previously (Ralevic *et al.*, 1994). 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 × 5 cm) in a humid chamber (custom made at University College London). The preparation was perfused at a constant flow rate of 5 ml min^{-1} by a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, Illinois).The perfusate was 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 P23XL, Viggo-Spectramed, Oxnard, CA) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument, Co., Quincy, Mass). Preparations were allowed to equilibrate for 30 min before experimentation.

Stimulation of perivascular nerves

Electrical stimulation (stimulator model SD9, Grass) of perivascular nerves was achieved by passing a current across the preparation between the cannulation needle and the wire grid on which the preparation rested. Stimulation at basal tone (90 V, 1 ms, 4–32 Hz, for 30 s) elicited vasoconstrictor responses which could be abolished by guanethidine (3 μM), confirming that these resulted from stimulation of perivascular sympathetic nerves.

Experimental protocol

A mesenteric arterial preparation from a vitamin E-deficient rat was tested simultaneously with that of an age-matched control. Perivascular nerve stimulation at a range of frequencies (4–32 Hz) was carried out to allow frequency-response curves to be constructed. Vasoconstrictor responses to doses of the sympathetic transmitter noradrenaline (NA) were then assessed. The tone of the preparations was raised by continuous perfusion with methoxamine (4–70 μM) to allow vasodilator responses to doses of acetylcholine (ACh), ATP and sodium nitroprusside (SNP) to be established. Responses of preparations to a dose of KCl (0.15 mmol) were determined at the end of experiments after all drugs had been washed out and after equilibration at basal tone for 15 min.

Drugs

All drugs were applied as 50 μl bolus injections into a rubber septum proximal to the preparation unless otherwise stated. Drugs were made up daily in distilled water except for NA which was made up as a stock solution of 10 mM in 0.1 mM ascorbic acid. Noradrenaline (arterenol bitartrate), methoxamine hydrochloride, adenosine 5′-triphosphate (disodium salt), acetylcholine chloride and sodium nitroprusside were all obtained from Sigma, Poole, U.K.

Data analysis

Responses were measured as changes in perfusion pressure (mmHg) and results presented as the mean ± s.e.mean. The relationships between the frequency- and dose-responses of the groups with diet were tested by analysis of variance with repeated measures, followed by Student's *t* test to see where the differences lay. Differences between means were considered significant when $P < 0.05$.

Results

Animals

The mean weights (g) of control and vitamin E-deficient rats at each age are given in Table 1. Vitamin E-deficient rats were significantly heavier ($P < 0.05$) than age-matched controls at 4 months. At 12 months, vitamin E-deficient rats were significantly lighter than age-matched controls ($P < 0.05$). After 12 months on a vitamin E-deficient diet rats exhibited poor coat condition, muscle wasting, kyphoscoliosis, hind limb weakness and impaired gait.

Table 1 Base-line parameters for mesenteric arterial preparations from control and vitamin E-deficient rats with age

	Age (months) 2	4	6	10	12
Body weights					
Control	333 ± 8 (6)	413 ± 16 (6)	512 ± 10 (6)	594 ± 25 (8)	696 ± 27 (15)
Vitamin E-deficient	330 ± 14 (6)	500 ± 23 (6)*	524 ± 31 (8)	554 ± 28 (8)	584 ± 14 (19)*
Basal perfusion pressure (mmHg)					
Control	49.0 ± 3.8 (6)	31.8 ± 3.1 (6)	31.9 ± 1.6 (6)	30.8 ± 2.1 (8)	26.4 ± 1.8 (15)
Vitamin E-deficient	48.3 ± 3.7 (6)	43.7 ± 3.7 (6)*	33.1 ± 1.9 (8)	30.5 ± 1.8 (8)	32.7 ± 1.7 (19)*
Increase in perfusion pressure (mmHg)[1]					
Control	84.0 ± 18.6 (5)	110.6 ± 5.0 (4)	83.9 ± 7.4 (5)	68.7 ± 6.6 (8)	100.1 ± 6.0 (5)
Vitamin E-deficient	74.5 ± 16.8 (6)	108.8 ± 9.7 (6)	73.1 ± 4.1 (6)	64.6 ± 5.3 (8)	89.5 ± 6.7 (10)
Concentration of methoxamine (μM)[2]					
Control	30 ± 0 (5)	35 ± 0.5 (4)	5.0 ± 0.8 (5)	59 ± 1.1 (8)	20 ± 0.7 (5)
Vitamin E-deficient	30 ± 0 (6)	37 ± 0.4 (6)	5.5 ± 0.5 (6)	55 ± 1.1 (8)	13.6 ± 0.5 (10)
Responses to KCl (mmHg)					
Control	71.4 ± 24 (5)	59.2 ± 17 (5)	57.5 ± 8 (6)	47.8 ± 5.2 (8)	50.0 ± 6 (12)
Vitamin E-deficient	79.0 ± 6 (4)	51.8 ± 14 (5)	40.3 ± 5 (8)	43.5 ± 5 (8)	52.4 ± 7 (10)

Data are shown are means ± s.e.mean
[1]Perfusion pressure raised with methoxamine
[2]Concentration of methoxamine used to raise the tone

Base-line parameters

The mean basal perfusion pressure (mmHg) of mesenteric arterial beds at each age for control and vitamin E-deficient rats respectively are given in Table 1. Basal perfusion pressure was significantly higher in mesenteric preparations from vitamin E-deficient rats than in controls at 4 months ($P<0.05$), and at 12 months ($P<0.05$).

At any given age there was no significant difference between mesenteric preparations from control and vitamin E-deficient groups with respect to the increase in tone produced by methoxamine, or in the concentration of methoxamine required to elicit this degree of tone. The increases in tone above baseline (mmHg) produced by methoxamine and the concentrations of methoxamine used to elicit this increase are given in Table 1.

Vasoconstrictor responses to stimulation of sympathetic nerves

Electrical field stimulation of perivascular nerves produced frequency-dependent vasoconstrictor responses due to activation of sympathetic nerves. Vasoconstrictor responses were of similar magnitude in mesenteric arterial beds from control and vitamin E-deficient rats at 2, 4, 6 and 10 months (Figure 1). At 12 months the frequency-response curves were significantly different between the groups ($P<<0.001$). At 24 and 32 Hz responses were significantly greater in vitamin E deficient rats than in age-matched controls ($P<0.01$) (Figure 1b).

Vasoconstrictor responses to exogenous NA and KCl

Bolus injections of NA elicited dose-dependent vasoconstrictor responses in the mesenteric arterial bed preparations. There was no difference between vitamin E-deficient and control groups with respect to responses to exogenous NA (Figure 2) at any age. There were no significant differences between vitamin E-deficient rats and age-matched control groups with respect to vasoconstrictor responses to KCl at any age (0.15 mmol) (Table 1).

Vasodilator responses to ACh, ATP and SNP

Relaxations to ACh were similar in mesenteric beds from vitamin E-deficient rats and their respective controls at 2, 4 and 6 months (Figure 3a). There was no significant group by dose effect for ACh-mediated relaxations of mesenteric beds from rats deprived of vitamin E for 10 and 12 months compared to the age-matched controls (Figure 3b).

At 12 months, but not at 10 months of vitamin E deficiency there was a significant impairment of dose-dependent relaxations to ATP ($P<0.05$) (Figure 4). There was a significant impairment of maximal relaxation at 5 nmol ATP ($P<0.05$). Vasodilator responses to SNP were not different betwen controls and vitamin E-deficient rats at any age (Figure 5).

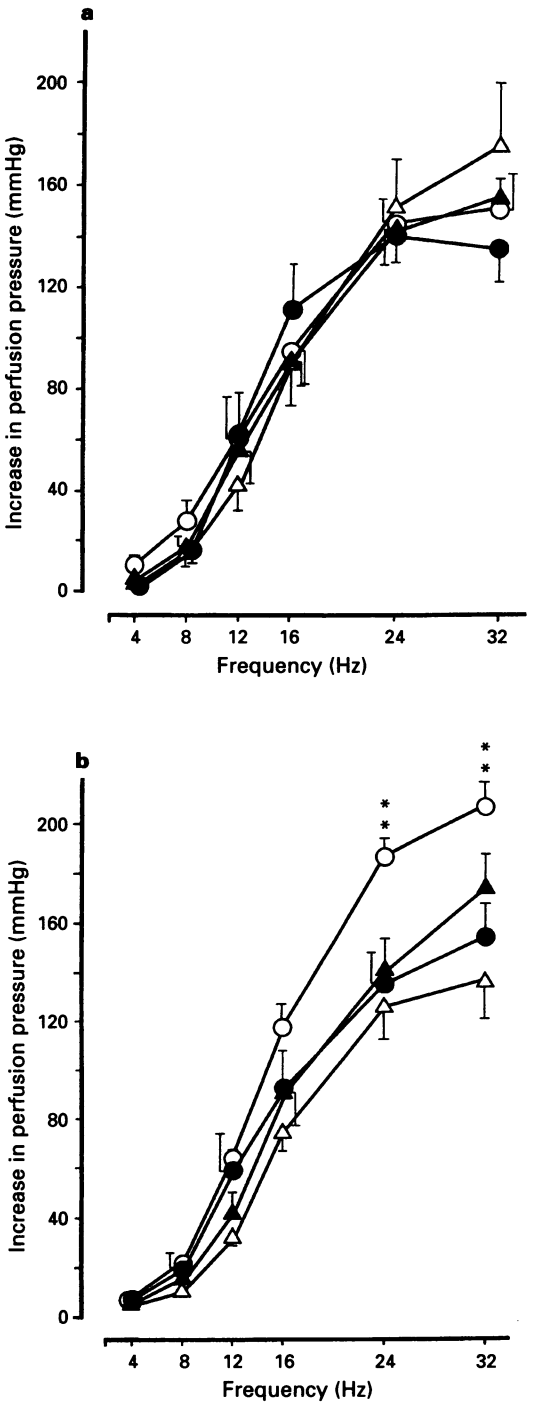

Figure 1 Frequency-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to electrical field stimulation (4–32 Hz, 90V 1 ms, for 30 s). (a) 4-month control (●, $n=6$); 4-month vitamin E-deficient (○, $n=6$); 6-month control (▲, $n=6$); 6-month vitamin E-deficient (△, $n=7$). (b) 10-month control (▲, $n=6$); 10-month vitamin E-deficient (△, $n=6$); 12-month control (●, $n=10$); 12-month vitamin E-deficient (○, $n=8$). Statistical difference from control is denoted by ** $P<0.01$. Vertical lines show s.e. mean.

Discussion

This study was designed to examine the function of sympathetic nerves, vascular smooth muscle and endothelium in mesenteric resistance blood vessels of the rat at different times of vitamin E deprivation to assess their relative susceptibilities to change in antioxidant stress. Animals on the vitamin E supplemented diet received 1–2 mg α-tocopherol daily (based on an approximate daily food intake of 10–20 g). This compares favourably with the current recommended daily intake of vitamin E of 12 mg for adult men and 10.3 mg for adult women (Simonoff *et al.*, 1992), and would suffice to prevent dietary vitamin E deficiency.

The lack of differences between the groups with respect to vasoconstriction to exogenously applied NA indicates that the enhanced sympathetically-mediated constrictor responses seen at 12 months after vitamin E deprivation were not due to changes in postjunctional mechanisms. Vitamin E deficiency is known to cause dying-back neuropathy, in which there is a decrease in anterograde and retrograde axonal transport. This principally affects the primary sensory axons (Muller & Goss-Sampson, 1990) and ultrastructural studies have shown enlargement of sensory axon terminals and accumulation of cytoplasmic organelles (Towfighi, 1981). It is possible that a similar accumulation of transmitter in the sympathetic nerve terminal may account for the increase in sympathetic responses

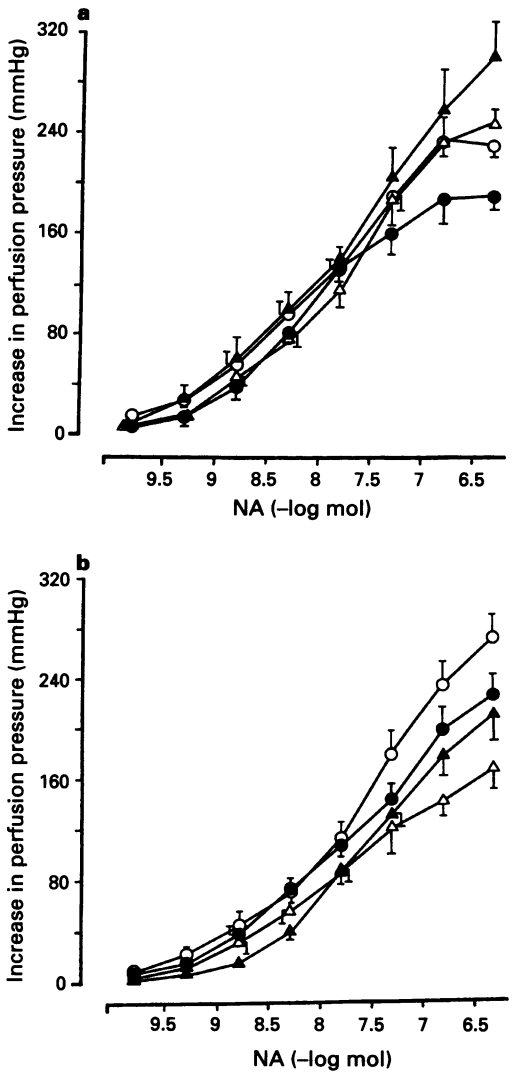

Figure 2 Dose-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to exogenous noradrenaline (NA). (a) 4-month control (●, $n=6$); 4-month vitamin E-deficient (○, $n=6$); 6-month control (▲, $n=6$); 6-month vitamin E-deficient (△, $n=8$). (b) 10-month control (▲, $n=6$); 10-month vitamin E-deficient (△, $n=6$); 12-month control (●, $n=10$); 12-month vitamin E-deficeint (○, $n=9$). Vertical lines show s.e. mean.

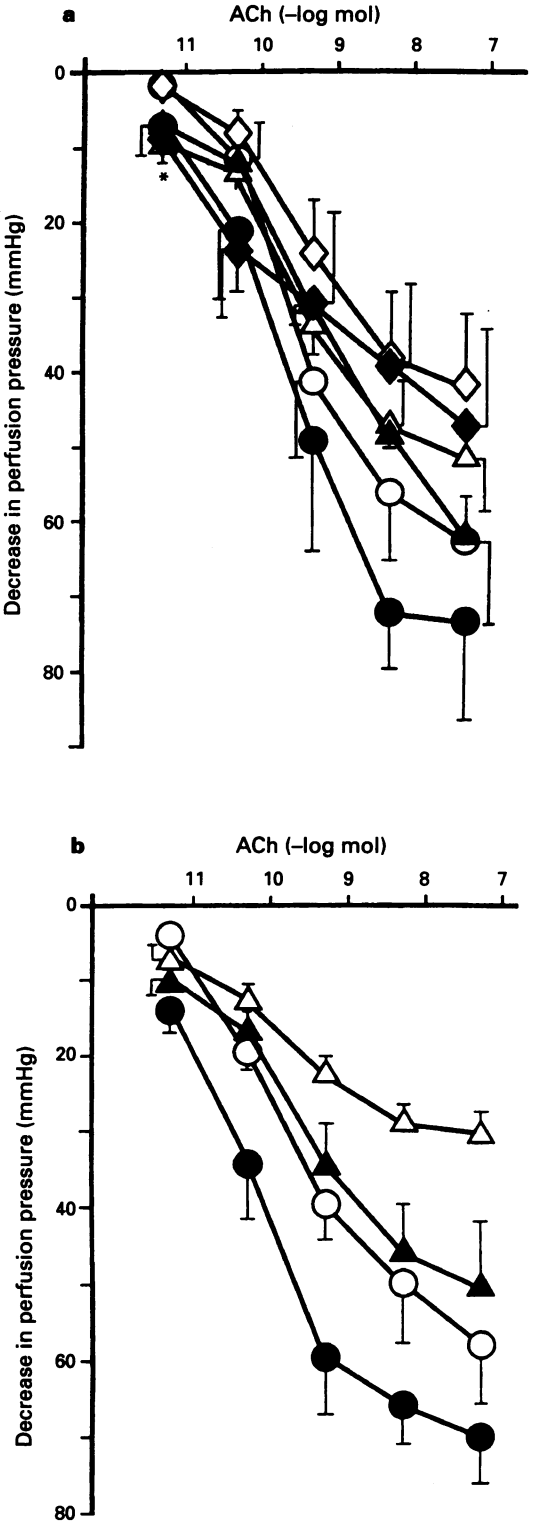

Figure 3 Dose-response curves showing vasodilator responses (decrease in perfusion pressure, mmHg) of rat mesenteric arterial beds to exogenous acetylcholine (ACh). (a) 2-month control (▲, $n=5$); 2-month vitamin E-deficient (△, $n=6$); 4-month control (●, $n=4$); 4-month vitamin E-deficient (○, $n=6$); 6-month control (◆, $n=5$); 6-month vitamin E-deficient (◇, $n=6$). (b) 10-month control (▲, $n=8$); 10-month vitamin E-deficient (△, $n=7$); 12-month control (●, $n=5$); 12-month vitamin E-deficient (○, $n=10$). Vertical lines show s.e. mean.

seen in the present study. Schmidt *et al.* (1991) have shown that chronic vitamin E deficiency results in the premature and exaggerated development of neuroaxonal dystrophy in primary sensory axon terminals in rat medullary gracile/cuneate nuclei. However, there was no such increase in the frequency of neuroaxonal dystrophy in the coeliac/superior mesenteric sympathetic ganglia (Schmidt *et al.*, 1991). A recent study showed that after 12 months of vitamin E deficiency there was dysfunction of non-adrenergic, non-cholinergic pre- and post-junctional autonomic transmission in the rat caecum, but no changes in sympathetic neuromuscular transmission in the rat urinary bladder (Hoyle *et al.*, 1995), suggesting that the autonomic neuropathy is not a generalized process. In addition to NA, transmitters such as ATP and neuropeptide Y (NPY) are known to be coreleased from sympathetic nerves, particularly at high frequencies of stimulation (Burnstock, 1990), and it is possible that the relative proportions of these transmitters may change in vitamin E deficiency.

Impaired relaxations to ATP in mesenteric arterial preparations from vitamin E-deficient rats at 12 months were a consequence of changes in the endothelium rather than in the smooth muscle since endothelium-independent relaxations to SNP were unaffected. In addition to endothelium-dependent vasodilatation, ATP elicits constriction via receptors on the smooth muscle. When endothelial function is impaired this may be augmented and may explain why a statistically significant difference in endothelium-dependent relaxation was observed for ATP but not for ACh.

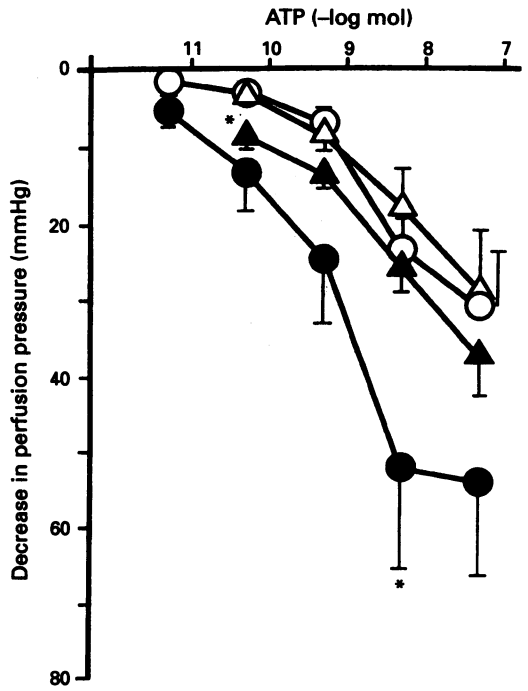

Figure 4 Dose-response curves showing vasodilator responses (decrease in perfusion pressure, mmHg) of rat mesenteric arterial beds to exogenous adenosine 5'-triphosphate (ATP). 10-month control (▲, $n=6$); 10-month vitamin E-deficient (△, $n=8$); 12-month control (●, $n=5$); 12-month vitamin E-deficient (○, $n=10$). Statistical difference is denoted by $^*P<0.05$. Vertical lines show s.e. mean.

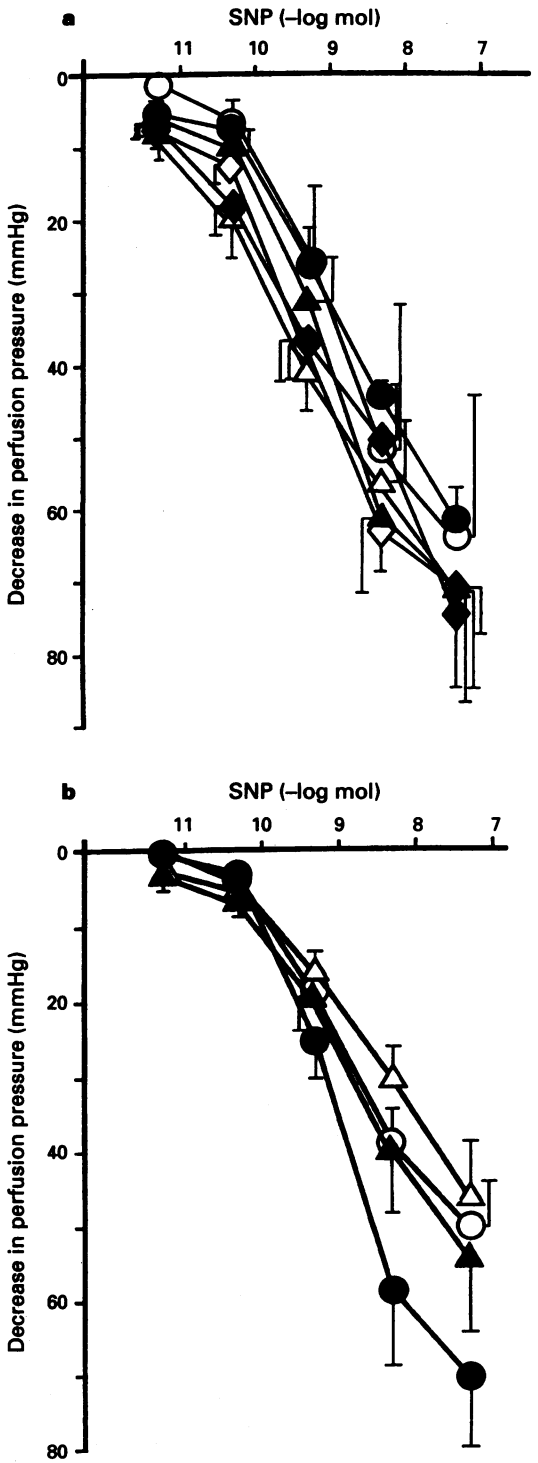

Figure 5 Dose-response curves showing vasodilator responses (decrease in perfusion pressure, mmHg) of rat mesenteric arterial beds to exogenous sodium nitroprusside (SNP). (a) 2-month control (▲, $n=5$); 2-month vitamin E-deficient (△, $n=6$); 4-month control (●, $n=4$); 4-month vitamin E-deficient (○, $n=6$); 6-month control (◆, $n=5$); 6-month vitamin E-deficient (◇, $n=6$). (b) 10-month control (▲, $n=7$); 10-month vitamin E-deficient (△, $n=8$); 12-month control (●, $n=6$); 12-month vitamin E-deficient (○, $n=11$). Vertical lines show s.e. mean.

While these results are consistent with earlier suggestions of a protective role of dietary vitamin E on mesenteric endo-thelial structure and function (Hubel *et al.*, 1989; Davidge *et al.*, 1993) the relative susceptibilities of the endothelium to impairment differs between the studies. In the present study differences were apparent at 12 months whereas Hubel and coworkers showed impaired ACh-mediated vasodilatation in the isolated superior mesenteric artery of rats at 35 weeks after dietary vitamin E deficiency, and provided morphological evidence for changes in the endothelium, manifested as surface 'blebbing', in femoral arteries from the same vitamin E-deficient animals (Hubel *et al.*, 1989). A recent study described impaired endothelium-dependent vasodilatation of the rat aorta 4 months after deprivation of dietary vitamin E (Rubino & Burnstock, 1993). The reason for these differences is not clear.

The reason why it takes several months of vitamin E deprivation for an alteration in vasomotor responses to become apparent may be related to the time for depletion of α-tocopherol from the tissues. In a study measuring the loss of α-tocopherol from tissues due to dietary vitamin E deficiency Goss-Sampson and colleagues found different rates of loss in different tissues (Goss-Sampson *et al.*, 1988). Both neural and non-neural tissues showed an initial rapid loss during the first 4 to 8 weeks of deficiency, followed by a second phase of slow prolonged depletion (Goss-Sampson *et al.*, 1988). It has been suggested that the first phase may correspond to a rapidly mobilized pool of labile vitamin E while the second phase represents α-tocopherol bound to subcellular or membraneous structures whose depletion relates to the loss of the functional and more critical components of tissue vitamin E (Bieri, 1972; Muller & Goss-Sampson, 1990,). In addition, a number of antioxidants are present in the circulation, for example, ascorbate, superoxide dismutase, erythrocytes and plasma proteins which may compensate to some extent for vitamin E deficiency, at least in the early stages.

Impaired endothelium-dependent vasodilatation in vitamin E deficiency may result from increased lipid peroxidation, causing destabilization of the endothelial cell membrane involving changes in fluidity, inactivation of membrane-bound receptors and enzymes, and increased non-specific permeability to ions such as Ca^{2+} (Halliwell & Chirico, 1993; Rice-Evans & Burdon, 1993). It is also possible that there are fewer intact healthy endothelial cells in vitamin E deficiency. Impaired endothelium-dependent responses to ATP seen in the present study are more likely to involve endothelium-derived

relaxing factor (EDRF)/nitric oxide (NO) than vasodilator products of the cyclo-oxygenase pathway since indomethacin has no effect on ATP-mediated relaxations in this preparation (unpublished observations). NO can be destroyed by generators of free radicals, and this effect can be prevented by free radical scavengers (Gryglewski *et al.*, 1986; Rubanyi & Vanhoutte, 1986). On the other hand, Davidge *et al.* (1993) have shown that after 10 weeks of vitamin E deficiency there is an increase in production of a vasoconstrictor product of the cyclo-oxygenase pathway in rat mesenteric arteries. Another mechanism which may contribute to endothelial malfunction in vitamin E deficiency involves the atherogenic effect on the endothelium by oxidised low-density lipoproteins (LDLs) which markedly increases if these become modified by peroxidation of their polyunsaturated fatty acids (PUFAs) (Esterbauer *et al.*, 1990; Gey *et al.*, 1991; Gey, 1992).

The higher basal perfusion pressure of mesenteric arterial beds from 12 month vitamin E-deficient rats may point to a loss of a continuous basal release of EDRF/NO. On the other hand, basal perfusion pressure at 4 months was also enhanced.

In conclusion, these results suggest that rat mesenteric arterial endothelial function is impaired due to antioxidant stress caused by vitamin E deficiency. At the luminal surface of blood vessels endothelial cells are in a prime position for attack by circulating free radicals. This may explain the relative resistance to dysfunction of the underlying vascular smooth muscle since endothelial cells may act as a barrier to circulating free radicals. Constrictor responses mediated by mesenteric sympathetic perivascular nerves are increased, and this does not appear to be due to postjunctional changes. Since the mesenteric circulation receives 15–20% of total cardiac output (Jacobson, 1982) and provides a major contribution to total peripheral resistance and capacitance, impaired endothelial mesenteric vascular control in vitamin E deficiency may compromise overall cardiovascular homeostasis, in addition to possible compromises in perfusion of the gastrointestinal tract.

The support of the British Heart Foundation is gratefully acknowledged.

References

BIERI, J.G. (1972). Kinetics of tissue alpha-tocopherol depletion and repletion. *Ann. NY Acad. Sci.*, **203**, 181–191.

BURNSTOCK, G. (1990). The Fifth Heymans Lecture, Ghent. Co-Transmission. *Arch. Int. Pharmacodyn. Ther.*, **304**, 7–33.

BURTON, G.W., JOYCE, A. & INGOLD, K.U. (1983). Is vitamin E the only lipid soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? *Arch. Biochem. Biophys.*, **221**, 281–290.

DAVIDGE, S.T., HUBEL, C.A. & McLAUGHLIN, M.K. (1993). Cyclo-oxygenase-dependent vasoconstrictor alters vascular function in the vitamin E-deprived rat. *Circ. Res.*, **73**, 79–88.

ESTERBAUER, H., DIEBER-ROTHENEDER, M., WEAG, G., STRIEGL, G. & JURGENS, G. (1990). Biochemical, structural and functional properties of oxidized low-density lipoprotein. *Chem. Res. Toxicol.*, **3**, 77–92.

GEY, K.F. (1992). Vitamin E and other essential antioxidants regarding coronary heart disease: risk assessment studies. A review. In *Vitamin E: Biochemistry and Clinical Applications*. ed. Packer, L. & Fuchs, J., pp. 589–633. New York: Marcel Dekker.

GEY, K.F., PUSKA, P., JORDAN, P. & MOSER, U.K. (1991). Inverse correlation between plasma vitamin E and mortality from ischaemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.*, **53**, 326S–334S.

GOSS-SAMPSON, M.A., KRISS, A. & MULLER, D.P.R. (1990). A longitudinal study of somatosensory, brainstem auditory and peripheral sensory-motor conduction during vitamin E deficiency in the rat. *J. Neurol. Sci.*, **100**, 79–84.

GOSS-SAMPSON, M.A., MACEVILLY, G.J. & MULLER, D.P.R. (1988). Longitudinal studies of the neurobiology of vitamin E and other antioxidant systems, and neurological function in the vitamin E deficient rat. *J. Neurol. Sci.*, **87**, 25–35.

GRYGLEWSKI, R.J., PALMER, R.M.J. & MONCADA, S. (1986). Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. *Nature*, **320**, 454–456.

HALLIWELL, B. & CHIRICO, S. (1993). Lipid peroxidation: its mechanism, measurement and significance. *Am. J. Clin. Nutr.*, **57**, 715S–725S.

HARDING, A. (1987). Vitamin E and the nervous system. *CRC Crit. Rev. Neurobiol.*, **3**, 89–103.

HOYLE, C.H.V., RALEVIC, V., LINCOLN, J., KNIGHT, G.E., GOSS-SAMPSON, M.A., MILLA, P.J. & BURNSTOCK, G. (1995). Effects of vitamin E deficiency on autonomic neuroeffector mechanisms in the rat caecum, vas deferens and urinary bladder. *J. Physiol.*, **487.3**, 773–786.

HUBEL, C.A., GRIGGS, K.C. & McLAUGHLIN, M.K. (1989). Lipid peroxidation and altered vascular function in vitamin E-deficient rats. *Am. J. Physiol.*, **256**, H1539–H1545.

JACOBSON, E.D. (1982). Physiology of the mesenteric circulation. *Physiologist*, **25**, 439–443.

KANEKO, T., NAKANO, S. & MATSUO, M. (1991). Protective effect of vitamin E on linoleic acid hydroperoxide-induced injury to human endothelial cells. *Lipids*, **26**, 345–348.

McGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.*, **177**, 21–30.

MULLER, D.P.R. & GOSS-SAMPSON, M.A. (1990). Neurochemical, neurophysiological, and neuropathological studies in vitamin E deficiency. *Crit. Rev. Neurobiol.*, **5**, 239–263.

OLSON, J.A. & KOBAYASHI, S. (1992). Antioxidants in health and disease. *Proc. Soc. Exp. Biol. Med.*, **200**, 245–247.

PACKER, L. (1992). Interactions among antioxidants in health and disease: vitamin E and its redox cycle. *Proc. Soc. Exp. Biol. Med.*, **200**, 271–276.

RALEVIC, V., RUBINO, A. & BURNSTOCK, G. (1994). Prejunctional modulation of sensory-motor nerve mediated vasodilatation of the rat mesenteric arterial bed by opioid peptides. *J. Pharmacol. Exp. Ther.*, **268**, 772–778.

RICE-EVANS, C. & BURDON, R. (1993). Free radical-lipid interactions and their pathological consequences. *Prog. Lipid. Res.*, **22**, 71–110.

RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol.*, **250**, H822–H827.

RUBINO, A. & BURNSTOCK, G. (1994). Recovery after dietary vitamin E supplementation of impaired endothelial function in vitamin E-deficient rats. *Br. J. Pharmacol.*, **112**, 515–518.

SCHMIDT, R.E., COLEMAN, B.D. & NELSON, J.S. (1991). Differential effect of chronic vitamin E deficiency on the development of neuroaxonal dystrophy in rat gracile/cuneate nuclei and prevertebral sympathetic ganglia. *Neurosci. Lett.*, **123**, 102–106.

SIMONOFF, M., SERGEANT, C., GARNIER, N., MORETTO, P., LLABADOR, Y., SIMONOFF, G. & CONRI, C. (1992). Antioxidant status (selenium, vitamins A and E) and aging. In *Free Radicals and Aging*. ed. Emerit, I. & Chance, B. pp. 368–397. Basel, Switzerland: Birkhäuser Verlag.

SOKOL, R.J. (1990). Vitamin E and neurologic deficits. *Adv. Pediatr.*, **37**, 119–148.

TOWFIGHI, J. (1981). Effects of chronic vitamin E deficiency on the nervous system of the rat. *Acta Neuropathol.*, **54**, 261–267.

VAN ACKER, S.A.B.E., KOYMANS, L.M.H. & BAST, A. (1993). Molecular pharmacology of vitamin E: structural aspects of antioxidant activity. *Free Radical Biol. Med.*, **15**, 311–328.

(Received January 5, 1995
Revised July 17, 1995
Accepted August 21, 1995)