Effect of chronic vitamin E deficiency on sympathetic and sensorimotor function in rat mesenteric arteries

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1. Mesenteric arterial beds from male rats deprived of vitamin E for 12 months postweaning were isolated and perfused at 5 ml min^{-1}.

2. The basal perfusion pressure of vitamin E-deficient preparations was significantly higher (34·0 ± 1·9 mmHg, $n = 15$) than in age-matched controls (26·1 ± 2 mmHg, $n = 14$; $P < 0·01$).

3. At basal tone, vasoconstrictor responses to electrical field stimulation (EFS) were not attenuated by vitamin E deficiency; at high stimulation frequencies, responses were enhanced. According to dose–response curves, exogenous noradrenaline was significantly more efficacious in preparations from vitamin E-deficient rats ($P < 0·05$).

4. In preparations with tone raised by methoxamine (6–20 μM) and in the presence of guanethidine (5 μM), EFS of perivascular sensorimotor nerves elicited frequency-dependent vasodilatation which was significantly attenuated by vitamin E deficiency. There was no difference in relaxation to calcitonin gene-related peptide (CGRP; $1·5 \times 10^{-11}$ mol), or to the sensory neurotoxin capsaicin (5×10^{-11} mol).

5. Immunohistochemical analysis of CGRP-containing nerves in the superior mesenteric artery showed no differences in density of innervation.

6. In conclusion, chronic vitamin E deficiency impairs sensorimotor vasodilatation in rat mesenteric arteries; this does not appear to be due to changes in postjunctional receptors, or to a depletion of transmitter (CGRP) content of the superior mesenteric artery. Sensorimotor nerves appear to be more vulnerable than sympathetic nerves to chronic vitamin E deficiency.

Vitamin E, comprising a family of naturally occurring lipid-soluble antioxidants (tocopherols and tocotrienols), is known to play a crucial role in the maintenance of normal membrane structure and function. This is effected principally via its chain-breaking antioxidant properties, thus protecting unsaturated fatty acids of membrane phospholipids from peroxidation, and also by modulation of signalling processes such as the formation of prostanoids, hydroxyeicosatetraenoic acid and sterols (Burton, Joyce & Ingold, 1983; Muller & Goss-Sampson, 1990; Olson & Kobayashi, 1992; Packer, 1992; Van Acker, Koymans & Bast, 1993). In addition, the phytyl side chain is thought to give vitamin E its membrane-stabilizing properties. Neural membranes appear to be particularly susceptible to the detrimental effects of oxidative processes and their impaired functioning in chronic vitamin E deficiency may result in ataxia, areflexia, pigmentary retinopathy and generalized muscle weakness (Muller, Lloyd & Wolff, 1983; Harding, 1987). Neuropathy due to vitamin E deficiency has been identified as a primary 'dying-back' axonopathy with secondary demyelination. This mainly affects primary sensory axons (Nelson, Fitch, Fischer, Brown & Chou, 1981; Towfighi, 1981), axonal degeneration being most apparent in the dorsal columns, especially in the gracile and cuneate nuclei of the spinal cord with peripheral nerves being less affected (Nelson *et al.* 1981; Nelson, 1987; Goss-Sampson, Kriss & Muller, 1990).

To date, much information on the mechanisms and consequences of vitamin E deficiency has come from electrophysiological studies on experimental vitamin E

deficiency in animals and in vitamin E-deficient patients. Relatively few studies have specifically examined the effects of vitamin E deficiency on vascular function and none has examined the functioning of perivascular nerves. The rat mesenteric arterial bed is a valuable model for study of the peripheral function of sympathetic and primary afferent nerves. Sympathetic vasoconstriction of rat mesenteric arteries is mediated by the postjunctional actions of the cotransmitters noradrenaline (NA) and ATP (Yamamoto, Cline & Takasaki, 1988; Sjöblom-Widfeldt, Gustafsson & Nilsson, 1990; Yamamoto, Takasaki & Nickols, 1992). Rat mesenteric arteries are also innervated by primary afferents which elicit capsaicin-sensitive vasodilatation due principally to the release of calcitonin gene-related peptide (CGRP) (Kawasaki, Takasaki, Saito & Goto, 1988; Fujimori, Saito, Kimura & Goto, 1990; Han, Naes & Westfall, 1990). Sensory afferents are activated by antidromic stimulation or by stimulation of the peripheral terminals to release a variety of peptide transmitters including substance P, neurokinin A and CGRP (Maggi & Meli, 1988). In recognition of their dual afferent and efferent functions the term 'sensory-motor (sensorimotor)' has been coined for these nerves (Burnstock, 1990). Particular susceptibility of sensorimotor nerves to impairment has been reported in ageing (Li & Duckles, 1993) as well as in certain disease states including hypertension (Kawasaki, Saito & Takasaki, 1990) and diabetes (Ralevic, Belai & Burnstock, 1993).

The aim of the present study was to examine the effects of long-term deprivation of vitamin E on sympathetic and sensorimotor nerve function in rat mesenteric arteries. Mesenteric arterial beds were isolated from rats deprived of dietary vitamin E for 12 months, a period of vitamin E deficiency known to result in peripheral sensory and motor neuropathies (Goss-Sampson *et al.* 1990; Muller & Goss-Sampson, 1990; Southam, Thomas, King, Goss-Sampson & Muller, 1991; Schmidt, Coleman & Nelson, 1991). The present study is the first direct assessment of the effects of vitamin E deficiency on sympathetic and sensorimotor neurotransmission in the vasculature.

METHODS

Animals and diet

Weanling (21–23 day), pathogen-free, male Wistar rats were obtained from Charles Rivers Limited, Kent, UK. One group of rats was placed on a diet deficient in vitamin E (vitamin-free casein, dextrose, stripped lard diet; Machlin/Draper-HLR no. 814, supplied by Dyets, Bethlehem, PA, USA) as previously described (Goss-Sampson *et al.* 1990). The vitamin E content of this diet was less than 1 ng ml^{-1}. Another group of rats served as controls, receiving the same diet to which alpha-tocopheryl acetate (40 mg $(kg\ diet)^{-1}$) was added. Food and water were provided *ad libitum*. Rats were used at 12 months.

Isolated mesenteric arterial bed preparation

Rats were killed by sodium pentobarbitone overdose (60 mg kg^{-1} I.P.). Mesenteric beds were isolated from male Wistar rats and set up for perfusion as described previously (Ralevic *et al.* 1993). 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} using a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, IL, USA). 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, USA) on a side-arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, MA, USA). Electrical stimulation of perivascular nerves was achieved by passing a current (stimulator model SD9, Grass) across the preparation between the cannulation needle and the wire grid on which the preparation rested. Preparations were allowed to equilibrate for 30 min prior to experimentation.

Experimental protocol

Experiments at basal tone. EFS at basal tone (4–32 Hz, 90 V, 1 ms, for 30 s) was carried out to allow frequency–response curves to be constructed. At these parameters of stimulation vasoconstrictor responses are abolished by guanethidine (5 μM) confirming that they are of sympathetic origin (Ralevic *et al.* 1993). Vasoconstrictor responses of preparations to doses of NA (50 μl containing $1·5 \times 10^{-10}$ to 5×10^{-7} mol) were then assessed. Responses of preparations to a single dose of KCl (0·15 mmol, approximately the ED_{25} dose) were determined at the end of these experiments to assess the responsiveness of the vascular smooth muscle to a non-receptor-mediated vasoconstrictor.

Raised-tone experiments. In separate experiments, mesenteric beds were treated as follows to allow selective EFS of sensorimotor nerves and recording of the resulting vasodilator response. After 10 min of equilibration, guanethidine (5 μM) was added to the perfusate to block sympathetic transmission and after a further 20 min the tone of the preparation was raised by adding methoxamine to the perfusate to a final concentration of 6–20 μM, in order to achieve an increase in perfusion pressure of 50–100 mmHg. EFS (60 V, 0·1 ms, 30 s duration, 1–12 Hz) then elicited vasodilator responses due to activation of primary sensory afferents and subsequent release of sensory transmitter (Kawasaki *et al.* 1988). These vasodilator responses are abolished by capsaicin treatment confirming their sensory origin (Kawasaki *et al.* 1988; Ralevic, Karoon & Burnstock, 1995). After an initial stimulation at a frequency of 8 Hz (to confirm continuity of the electrical circuit), vasodilator responses to activation of sensorimotor nerves at the full range of frequencies were established. The parameters of EFS which were used at basal and raised tone are optimal for producing guanethidine-sensitive constriction and capsaicin-sensitive vasodilatation, respectively.

Following EFS, responses of the preparations to doses (50 μl bolus injections) of exogenous CGRP ($1·5 \times 10^{-11}$ mol) and capsaicin

(5×10^{-11} mol) were established. These doses were chosen on the basis that they produce reproducible vasodilator responses of the same order of magnitude as those elicited by electrical stimulation. At the end of each experiment all drugs were washed out and the preparations allowed to equilibrate for 15 min at basal tone.

CGRP immunohistochemical analysis

Approximately 1 cm of the superior mesenteric artery was dissected from four control and four vitamin E-deficient rats. Vessels were cleaned, pinned out adventitial-side uppermost on Sylgard silicone rubber (Dow Corning, Poole, England) and stained for CGRP-like immunoreactivity (CGRP-LI) using a standard immunohistochemical method. Briefly, the vessels were fixed in 4% (w/v) paraformaldehyde in 0·1 M phosphate-buffered saline (PBS, pH 7·3) for 90 min. After washing in PBS (3 × 10 min) vessels were incubated with the primary rabbit antisera raised against CGRP at a dilution of 1 : 1000 for 16–18 h at room temperature. Vessels were then washed and incubated with biotinylated donkey anti-rabbit serum (1 : 250) for 90 min, and after washing again, were incubated with fluorescein isothiocyanate-conjugated streptavidin (1 : 250; Nordic Immunological Laboratories, Tilburg, The Netherlands) for 90 min at room temperature. The vessels were subsequently washed and counterstained with 0·05% Pontamine Sky Blue (BDH Chemicals Ltd, Poole, England) in PBS containing 1% dimethyl sulphoxide for 10 min, and mounted in Citifluor (City University, London). The immunolabelled tissues were examined under a Zeiss photomicroscope equipped for viewing fluorescein isothiocyanate fluorescence (Carl Zeiss, Inc., Thornwood, NY, USA) and with a KP560 filter to reduce the background levels stained by the Pontamine Sky Blue. Immunohistochemical procedures on vessels from control and vitamin E-deficient rats were carried out in parallel to ensure valid comparison between preparations. Control experiments to determine the specificity of antisera were carried out by preincubating CGRP antisera with antigen for CGRP. Immunoreactivity in the preparations was not seen when the primary antibody had been preincubated with CGRP (0·3 μM) for 1 h.

Drugs

NA, CGRP, capsaicin and KCl were applied as 50 μl bolus injections into a rubber septum proximal to the tissue. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) was initially made up as a stock solution of 10 mM in 100% ethanol and then diluted in distilled water. NA was made up as a stock solution of 10 mM in 0·1 mM ascorbic acid. All other drugs were made up in distilled water. Methoxamine hydrochloride, noradrenaline (arterenol bitartrate) and capsaicin were obtained from Sigma. Guanethidine monosulphate (Ismelin) was from Ciba-Geigy, Horsham, West Sussex, UK. CGRP and primary antisera for CGRP were from Cambridge Research Biochemicals Ltd, Cambridge, UK.

Data analysis

Responses were measured as changes in perfusion pressure (mmHg) and results presented as the mean ± S.E.M. Curves were compared by analysis of variance with repeated measures, with *post hoc* Students *t* test to determine where the differences lay; differences were considered significant when $P < 0·05$.

RESULTS

Animals

Rats which had been on the vitamin E-deficient diet for 12 months following weaning weighed significantly less (591 ± 16 g, $n = 15$) than the age-matched controls (692 ± 29 g, $n = 14$; $P < 0·001$). The vitamin E-deficient rats exhibited characteristic poor coat condition, muscle wasting, kyphoscoliosis, hindlimb weakness and impaired gait as described previously (Muller & Goss-Sampson, 1990).

Baseline parameters

The mean basal tone of mesenteric arterial preparations from vitamin E-deficient rats, 34·0 ± 2 mmHg ($n = 15$), was significantly higher than that of controls 26·1 ± 2 mmHg ($n = 14$, $P < 0·01$). There was no difference between the preparations in ability to constrict to 0·15 mmol KCl when this was applied at the end of basal-tone experiments as a test of the responsiveness of the vascular smooth muscle to a receptor-independent vasoconstrictor; responses to KCl produced an increase in perfusion pressure above baseline of 51·6 ± 7 mmHg

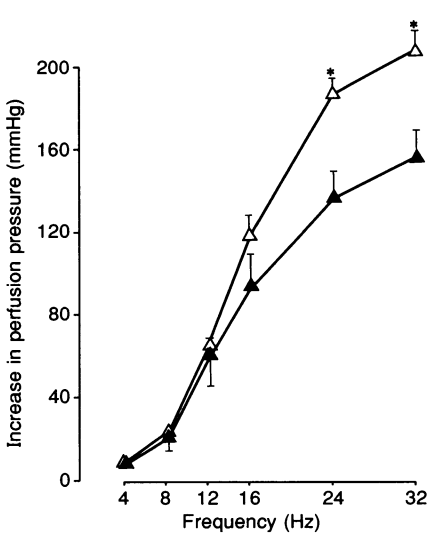

Figure 1. Effect of vitamin E deficiency on frequency–response curves of mesenteric arterial preparations

Frequency–response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to electrical field stimulation of sympathetic nerves (4–32 Hz, 90 V, 1 ms, for 30 s). ▲, 12-month control ($n = 10$); △, 12-month vitamin E deficient ($n = 8$). * Significantly different from control ($P < 0·01$).

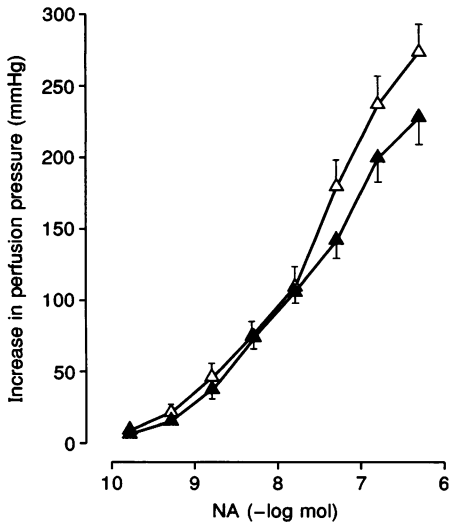

Figure 2. Dose–response curves to NA in control and vitamin E-deficient mesenteric arterial preparations

Dose–response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to noradrenaline (NA). ▲, 12-month control ($n = 10$); △, 12-month vitamin E deficient ($n = 9$). The curves were significantly different (ANOVA with repeated measures; $P < 0{\cdot}05$).

($n = 11$) in controls and $52{\cdot}4 \pm 7$ mmHg ($n = 10$) in vitamin E-deficient rats.

Vasoconstrictor responses to electrical field stimulation

EFS elicited frequency-dependent vasoconstrictor responses which were rapid in onset and which were maintained for only as long as the stimulation (30 s). Curves were significantly greater in mesenteric preparations of vitamin E-deficient rats compared with age-matched controls (ANOVA with repeated measures; $P < 0{\cdot}001$; Fig. 1). At individual frequencies the difference between the groups was greatest at the highest frequencies and reached statistical significance at 24 and 32 Hz ($P < 0{\cdot}01$; Fig. 1).

Vasoconstrictor responses to noradrenaline

Bolus injections of NA elicited dose-dependent vasoconstrictor responses in the mesenteric arterial bed preparations. Curves compared using ANOVA with repeated measures were found to be significantly different ($P < 0{\cdot}05$), although there was no significant difference in vasoconstrictor responses between the preparations at any given dose of NA (Fig. 2).

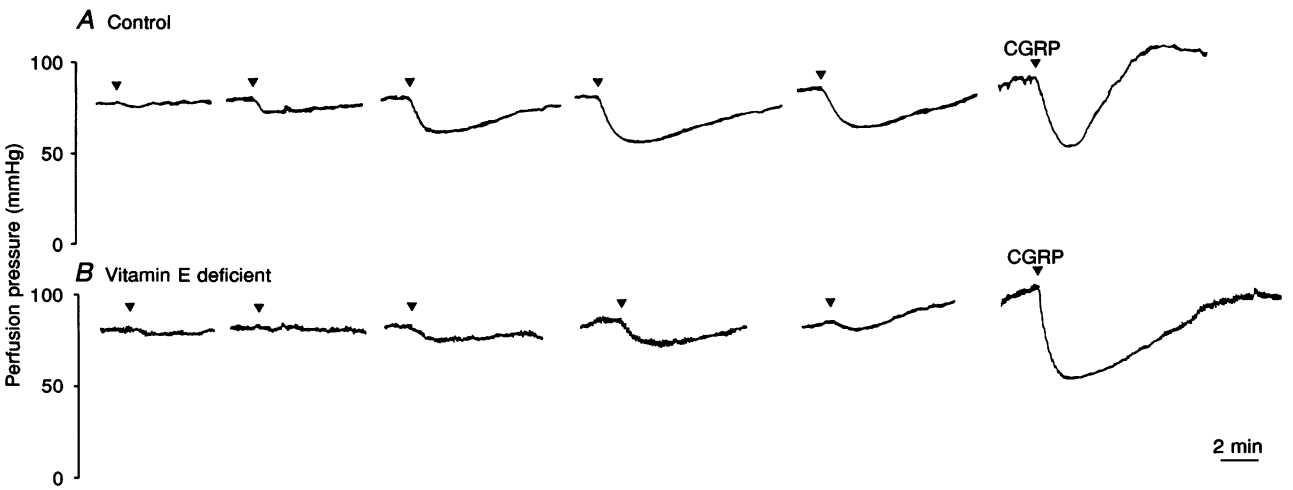

Figure 3. Effect of vitamin E deficiency on sensorimotor vasodilatation of mesenteric arterial preparations

Representative traces showing frequency-dependent vasodilator responses (decreases in perfusion pressure, mmHg) of mesenteric arterial beds from one control (*A*) and one 12-month vitamin E-deficient rat (*B*). Electrical field stimulation of sensorimotor nerves (▼, 1–12 Hz, 60 V, 0·1 ms, for 30 s) was carried out in the presence of guanethidine (5 μM) to block sympathetic neurotransmission and methoxamine (6–20 μM) to raise the tone of the preparations. Vasodilator responses to doses of calcitonin gene-related peptide (CGRP; $1{\cdot}5 \times 10^{-11}$ mol) were of similar magnitude in mesenteric beds from control and vitamin E-deficient rats.

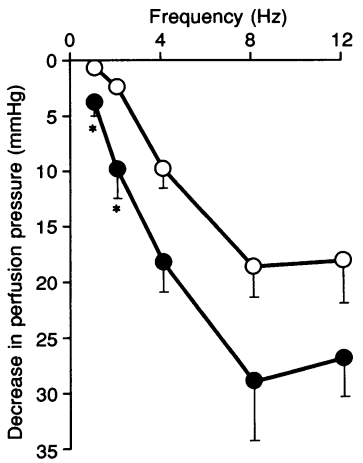

Figure 4. Effect of vitamin E deficiency on sensorimotor vasodilatation of mesenteric arterial preparations

Frequency–response curves showing mean vasodilator responses (decrease in perfusion pressure, mmHg) of mesenteric arterial beds from control (●, $n = 4$) and 12-month vitamin E-deficient (○, $n = 6$) rats. Sensorimotor nerve stimulation (1–12 Hz, 60 V, 0·1 ms, for 30 s) was carried out in the presence of guanethidine (5 μM) and methoxamine (6–20 μM). * Significantly different from control ($P < 0{\cdot}05$).

Vasodilator responses to electrical field stimulation of sensorimotor nerves

Adding methoxamine (6–20 μM) raised the tone of the preparations by $64{\cdot}3 \pm 5{\cdot}9$ mmHg ($n = 4$) and $64{\cdot}7 \pm 7{\cdot}4$ mmHg ($n = 6$) in mesenteric arterial beds from control and vitamin E-deficient rats, respectively. There was no significant difference between the groups with respect to the amount of tone produced in the raised-tone preparations. Although the preparations had individual requirements for the concentration of methoxamine required to raise the tone, there was no significant difference between the groups in the concentration of methoxamine that was used.

EFS for 30 s elicited frequency-dependent vasodilator responses which were characteristically slow in onset and long-lasting (Fig. 3). Maximal relaxation was achieved at 1–2 min after onset of stimulation and the response did not return to the preconstricted level of tone for up to 10 min at the highest frequencies. Vasodilator response curves in vitamin E-deficient preparations were lower than those of control preparations and this reached statistical significance at the lower end of the response curve, i.e. when curves were compared at frequencies of 1 and 2 Hz (ANOVA with repeated measures; Figs 3 and 4). *Post hoc* analysis showed significant differences at both of these individual frequencies ($P < 0{\cdot}05$). ANOVA showed no significant differences between the curves when higher frequencies were included in the analysis.

Vasodilator responses to exogenous CGRP and capsaicin

There were no significant differences between the groups with respect to their ability to relax to injections of CGRP ($1{\cdot}5 \times 10^{-11}$ mol) or capsaicin (5×10^{-11} mol; Fig. 5).

Immunohistochemical analysis of CGRP-LI mesenteric perivascular nerves

Immunohistochemical analysis of CGRP-LI in perivascular nerves of superior mesenteric arteries from control and vitamin E-deficient rats showed no obvious differences in the density of innervation by this population of nerve fibres (Fig. 6). Furthermore, the intensity of CGRP immunofluorescence was similar in mesenteric arteries from each of the two groups (Fig. 6).

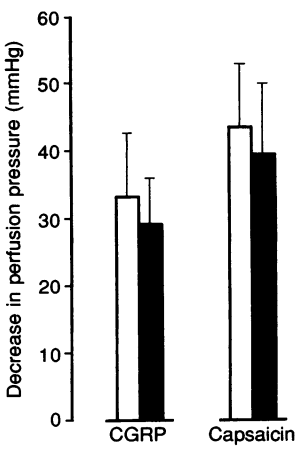

Figure 5. Vasodilatation to CGRP and capsaicin in control (□) and vitamin E-deficient (■) mesenteric arterial preparations

Histogram showing mean vasodilator responses of mesenteric arterial beds from control ($n = 4$) and vitamin E-deficient ($n = 5$) rats to doses of calcitonin gene-related peptide (CGRP; $1{\cdot}5 \times 10^{-11}$ mol) and capsaicin (5×10^{-11} mol).

DISCUSSION

The present study provides a functional correlate for biochemical, electrophysiological and morphological studies describing neural abnormalities associated with chronic vitamin E deficiency (see Muller & Goss-Sampson, 1990). Our results show that after 12 months of vitamin E deficiency there is impairment of sensorimotor neurotransmission in mesenteric arteries of the rat, manifested as a decrease in sensorimotor vasodilatation.

The absence of changes in the vasodilator action of exogenous CGRP, the principal vasodilator transmitter of sensorimotor nerves in rat mesenteric arteries (Kawasaki *et al.* 1988) indicates that vitamin E-deprivation has an adverse effect on tissue content and/or release of sensorimotor neurotransmitter(s) rather than on postjunctional receptors for CGRP, or second messenger systems involved in CGRP-mediated vasodilatation. However, we cannot exclude the possibility that changes in postjunctional

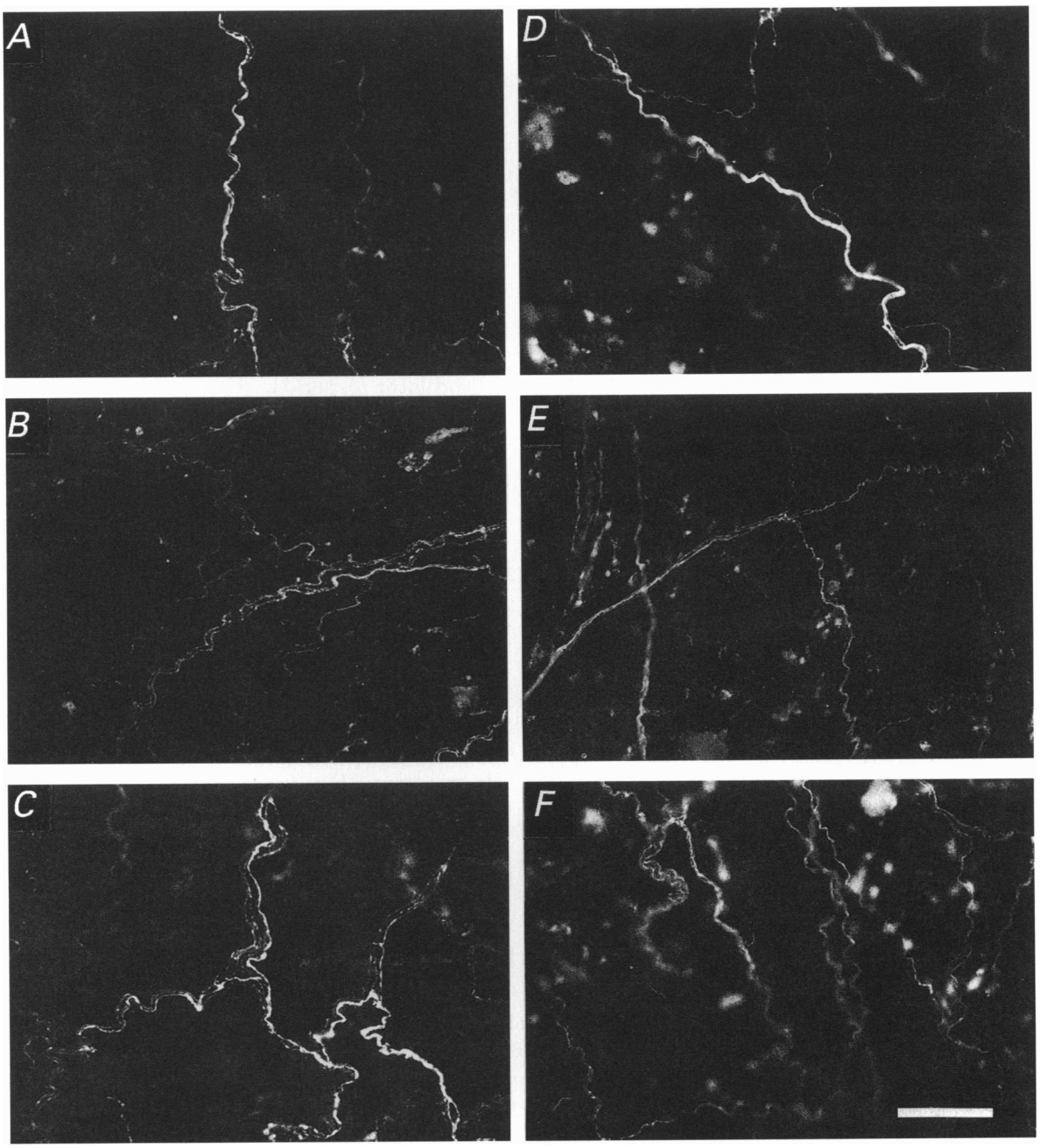

Figure 6. CGRP immunoreactivity in mesenteric arteries from control and vitamin E-deficient rats
Micrographs of CGRP immunoreactivity in the superior mesenteric artery of vitamin E-deficient (*A*, *B* and *C*) and control (*D*, *E* and *F*) rats. Panels *A* and *D* are from the proximal region of the vessel; panels *B* and *E* are from the mid-region, and panels *C* and *F* are from the distal region. Scale bar represents 50 μm.

mechanisms contributed to the decreased relaxations at lower frequencies of stimulation, since the greatest reduction in sensory vasodilatation was observed at the lowest frequencies. The lack of differences in responses to capsaicin, which excites sensory nerves via specific receptors coupled to non-selective cation channels (see Maggi & Meli, 1988), indicates that vitamin E deficiency affects mechanisms identical or analogous to those involved in antidromic stimulation and generation of the action potential.

Similar patterns of innervation density and intensity of fluorescence of CGRP-LI nerves in the superior mesenteric artery of vitamin E-deficient and control rats may suggest that impaired sensorimotor neurotransmission is a consequence of aberrant mechanisms of release rather than decreased levels of neurotransmitter stored in the nerves. On the other hand, innervation of the superior mesenteric artery may not be representative of that of the terminal arterioles which contribute importantly to the resistance of the whole-bed mesenteric arterial preparation. In the diabetic gut, despite the accumulation of vasoactive intestinal polypeptide (VIP)-LI in the enteric neurones and nerve fibres of 8-week streptozotocin-diabetic rat intestine (Belai, Lincoln, Milner, Crowe, Loesch & Burnstock, 1985; Belai, Lincoln, Milner & Burnstock, 1988), electrical stimulation of the enteric nerves did not induce an increase in VIP release (Belai, Lincoln & Burnstock, 1987). Clearly, malfunctions in neurotransmission should not be considered solely in terms of reductions of transmitter content of the innervated organ, since they may also be caused by defective release mechanisms even when the transmitter content has not altered or has increased. The mechanism by which vitamin E deficiency results in impaired neurotransmitter release may involve increased lipid peroxidation of the neural membrane and altered membrane fluidity, inactivation of membrane-bound receptors and enzymes, and an increase in non-specific permeability to ions such as Ca^{2+} (Halliwell & Chirico, 1993).

Other vasodilator peptides, including substance P, neurokinin A and VIP are known to be contained within and released from sensorimotor nerves (Maggi & Meli, 1988). However, in rat mesenteric arteries CGRP is known to be the principal vasodilator transmitter: capsaicin-sensitive vasodilator responses to EFS are blocked by a CGRP receptor antagonist and by CGRP receptor desensitization (Han *et al.* 1990); exogenously applied CGRP mimics the vasodilator response produced by electrical stimulation (Kawasaki *et al.* 1988); substance P and VIP have virtually no vasodilator effects, or are active only at high concentrations (Kawasaki *et al.* 1988); CGRP, but not other putative sensory transmitters, is released by EFS and by capsaicin treatment (Fujimori *et al.* 1990; Manzini *et al.* 1991). Thus, it is likely that the decrease seen in sensorimotor nerve function in long-term vitamin E deficiency can largely be attributed to changes involving CGRP.

There was no evidence for attenuation of sympathetic neurotransmission due to vitamin E deficiency at stimulation frequencies of up to 16 Hz, indeed vasoconstrictor responses were enhanced at the highest frequencies of stimulation (24 and 32 Hz). It is possible that the increased sympathetic constriction is the consequence of the removal of the opposite vasodilator response produced by sensorimotor nerves normally present under control conditions. Indeed, capsaicin treatment *in vitro* and *in vivo* has been shown to augment sympathetic constrictor responses in rat mesenteric arteries (Li & Duckles, 1992; Ralevic *et al.* 1995). If impaired sensorimotor nerve function is responsible for the increase in constrictor responses to EFS this effect appears to be proportionately greater at high frequencies of stimulation, although exactly why this should be so is not clear. It is also possible that the higher sympathetic responses are due to accumulation of transmitter in the nerve terminal due to impaired retrograde transport (Goss-Sampson, MacEvilly & Muller, 1988; Muller & Goss-Sampson, 1990). Diabetes, also causing dying-back neuropathy, has been associated with an increase in the levels of CGRP and VIP in rat skin and blood vessels of the lip, respectively (Karanth, Springall, Francavilla, Mirrlees & Polak, 1990). Whether the increase in sympathetic responses seen in the present study is associated with changes in the proportion of the coexisting transmitters NA, ATP and NPY is not clear.

The difference in responses to exogenous NA suggests that postjunctional changes may contribute to the increase in sympathetic vasoconstriction in vitamin E deficiency. Impairment of endothelium-dependent vasodilatation of rat mesenteric arteries in vitamin E deficiency (Hubel, Griggs & McLauglin, 1989; V. Ralevic, unpublished observations) would result in augmentation of sympathetic vasoconstrictor responses. On the other hand, our results, showing similar vasoconstrictor responses to exogenous KCl in mesenteric beds from vitamin E-deficient and control groups, indicate an absence of functionally represented structural changes in the blood vessel wall. This is consistent with the results of a previous study reporting no changes in contractile responses to KCl and calcium in isolated segments of rat mesenteric arteries after vitamin E deficiency (Hubel *et al.* 1989). Resistance of sympathetic transmission mechanisms to adverse effects of vitamin E deficiency also occurs in the vas deferens, at a time when enteric transmission (both cholinergic and purinergic) is distinctly impaired (Hoyle *et al.* 1995).

It is apparent that vitamin E deficiency affects mesenteric sympathetic and sensorimotor function in different ways. A different susceptibility of sympathetic and sensorimotor nerves to neuropathy has been observed in the rat mesenteric arterial bed in streptozotocin-diabetes where sensorimotor neurotransmission was impaired at a stage when sympathetic vasoconstriction remained intact (Ralevic *et al.* 1993). The importance of membrane stabilization for neural tissues has been suggested since the extended

membrane surface area and remoteness of the nerve ending from the cell body, both characteristic features of neuronal structure, increase their susceptibility to oxidative injury (Cavanagh, 1984; Muller & Goss-Sampson, 1990). In this respect, sensory nerves may be particularly vulnerable compared to sympathetic nerves because of the long axons arising from dorsal sensory ganglia. Previous reports have confirmed the susceptibility of sensory nerves to malfunction in animal models of vitamin E deficiency as well as in vitamin E-deficient man (Muller & Goss-Sampson, 1990). Ultrastructural studies by 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. Significantly, the frequency of neuroaxonal dystrophy failed to show a similar increase in severity in the coeliac/superior mesenteric sympathetic ganglion (Schmidt *et al.* 1991). In addition, fibre spectrum analysis has shown a loss of large-calibre myelinated sensory axons that was confined to the distal ends of axons in peripheral nerves such as the ulnar and sural nerves (Rosenblum, Keating, Prensky & Nelson, 1981; Wichman, Buchthal, Pezeshkpour & Gregg, 1985).

In conclusion, the results of the present study provide, for the first time, evidence for defective sensorimotor neurotransmission in mesenteric arteries of chronic vitamin E-deficient rats. The implication of these findings is that vitamin E is essential for the maintenance of the integrity of neuronal cell membranes, such that release of transmitter can take place. Further, the results suggest that sensory nerves are relatively more sensitive to vitamin E deficiency compared to sympathetic nerves. This may contribute to our understanding of the consequences of abnormal antioxidant status such as may occur in ageing or in certain diseases.

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