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α-Tocopherol as agonist in hypoxia

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In hypoxia, but not normoxia, α-tocopherol (vitamin E) acts as an agonist in guinea-pig isolated colon, producing dose-dependent increases in contractile activity. This effect is mimicked by agents, vitamin K_1 and phytol, which contain a structural similarity to the phytyl side chain of α-tocopherol, but is antagonized by vitamin K_3 which has a structure similar to the chromane ring of vitamin E. All of the agonist responses were blocked by atropine and potentiated by physostigmine but were unaffected by hexamethonium. However, responses to acetylcholine were not antagonized by vitamin K_3 and these data suggest that α-tocopherol, phytol, vitamins K_1 and K_3 may be acting on a 'hypoxia receptor' which mediates release of acetylcholine onto muscarinic receptors.

α-Tocopherol is a lipid soluble, antioxidant vitamin that can protect animals from the lethal effects of hypoxia (Telford et al 1954) and anoxia (Hove et al 1945). In-vitro, hypoxia-induced mechanical and biochemical changes in rabbit perfused heart (Guarnieri et al 1978), guinea-pig atria (Garnett & Todd 1983) and portal vein (Kelly & Richardson 1981) are attenuated by this vitamin. The locus of action of this protection is uncertain but is suggested to be on mitochondrial function (Nayler 1978) or RNA synthesis (Guarnieri et al 1980) in the heart and on anaerobic metabolism in the portal vein (Kelly et al 1982).

However, although these studies have demonstrated protection from the deleterious effects of exposure to hypoxia, a quantitative pharmacological analysis of the phenomenon has proved difficult, because direct, easily measurable responses to α-tocopherol have not been found. This study has therefore investigated a model system which allows direct measurement of responses to α-tocopherol in a simple in-vitro situation.

Methods

Small segments, 2 cm long, of transverse colon from the guinea-pig were suspended vertically in tissue baths containing Krebs-Henseleit solution bubbled with a 95% O_2/5% CO_2 gas mixture. Spontaneous activity in the colon was measured isometrically, using a force displacement transducer, and recorded on a Bioscience oscillograph. After a period of equilibration in normoxic conditions, the tissues were exposed to a hypoxic environment by bubbling the suspending Krebs-Henseleit solution with a 95% N_2/5% CO_2 gas mixture. The oxygen tension of the suspending solution was determined, using a Rank oxygen electrode linked with a Servoscribe potentiometric recorder, by comparison with a sucrose Tris buffer of known oxygen content.

The lipid soluble drugs used, D-α-tocopherol-type V, vitamin K_1 (phytomenadione), vitamin K_3 (menaphthone) and phytol, were dissolved in the solvent, dimethyl sulphoxide (DMSO), and made up freshly before each experiment. The water-soluble drugs, acetylcholine chloride, physostigmine (eserine), hexamethonium, mepyramine, methysergide and atropine were stored frozen and prepared freshly each week. All chemicals were supplied by Sigma Chemical Company Ltd.

Results and discussion

To test whether hypoxic conditions had been achieved in the tissue baths during the experiments, samples of Krebs-Henseleit solution were taken from the bath over a timed period from the start of bubbling with the 95% N_2/5% CO_2 gas mixture. The mean oxygen content measured in normoxic conditions was 4·26 μM in the 30 ml baths. Samples taken after 0·5, 2, 5, 10 and 15 min showed reductions, respectively, of 0·78, 95, 99 and 100% from the normoxic level.

Activity in the colon was monitored in both normoxic and hypoxic conditions. Spontaneous contractions, having an average frequency of 9 min^{-1} (range 6–12) and magnitude of 1·3g (range 0·9–1·7), declined in hypoxia to virtually zero with a concomitant drop in basal tone. The time taken for this decline to appear was 4–5 min and therefore corresponded to a measured reduction in oxygen level in the bathing solution approaching 95%.

In order to establish whether α-tocopherol had a protective effect on the colon the vitamin was incubated with the tissue for 10 min in normoxic conditions before the change to hypoxia. α-Tocopherol (150 μM) did not affect the rate or magnitude of spontaneous contractions in normoxic conditions nor did it affect basal tone. However, it significantly delayed the onset of the effects of hypoxia (Table 1).

The results confirmed the protective action of α-tocopherol found in other tissues. However, when the vitamin was added to the bath after hypoxic conditions had been established and spontaneous contractions in the colon had died away, it produced stimulation of muscle activity. The magnitude of this response was concentration dependent within the range 0·2–200 μM (Fig. 1).

The phenomenon was investigated further by examining responses to chemically similar molecules. α-Toco-

Table 1. Measurement of time taken for the appearance of the effects of hypoxia.

Control	Solvent (DMSO + Soybean oil)	α-Tocopherol (150 μM)
209·5 ±13·9 (21)	272·0 ±32·0 (5)	406·6 ±52·2 (6)

Figures are mean times (in seconds from onset of 95% N_2/5% CO_2) ± standard errors with numbers of animals used in brackets.

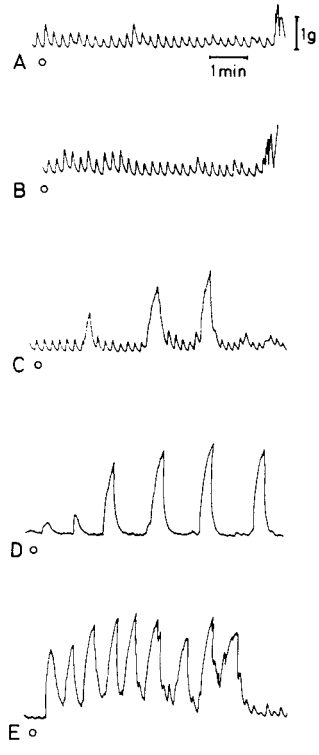

FIG. 1. Effect of increasing concentrations of α-tocopherol (O) on contractility of guinea-pig isolated colon after hypoxic conditions had been established. Key: A solvent; B 0·2 μM; C 2 μM; D 20 μM; E 200 μM α-tocopherol.

pherol is known to interfere with the coagulation activity of vitamin K (Woolley 1945; Korsan-Bengsten et al 1974; Corrigan & Marcus 1974). This and the structural similarity between the molecules suggested that this vitamin too might have agonist activity in the system.

Vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) showed no measurable effect on the spontaneous activity of the colon in normoxic conditions but in hypoxic conditions produced a similar effect to α-tocopherol in the same concentration range. To establish which portion of the molecule might be responsible for eliciting contractile response in hypoxia, two further compounds were used.

Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) is similar in length and structure to the side chains of the vitamin molecules. In the colon preparation phytol elicited contractile responses in hypoxic conditions which closely resembled those found with α-tocopherol and vitamin K_1 and again had no effect on the spontaneous contractions in normoxia.

Vitamin K_3 (menadione, 2-methyl-1,4-naphthoquinone) is the aromatic ring portion of vitamin K_1 but had no agonist activity in the colon. However, this compound was able to antagonize the contractions produced, in hypoxic conditions, by α-tocopherol, vitamin K_1 and phytol when added 5 min beforehand. Therefore vitamin K_3, the compound with the ring structure, appeared to be an antagonist of responses elicited by those compounds with the phytyl side chain. In three experiments the maximum responses to α-tocopherol, vitamin K_1 and phytol were reduced by vitamin K_3, indicating a degree of non-competitive antagonism. The results are summarized in Fig. 2.

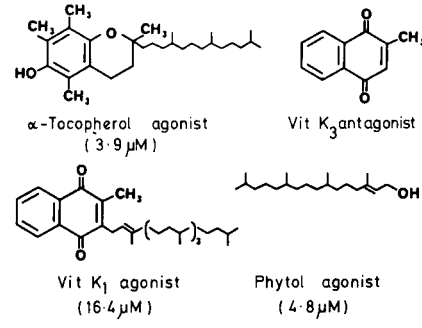

FIG. 2. Structure and activity of agonists and antagonist in colon in hypoxic conditions. Numbers in brackets are mean EC50 values for the agonists for five guinea-pigs.

The mechanism of the response was investigated by using some known agonists and antagonists of contractile responses in intestinal smooth muscle. Responses to α-tocopherol, vitamin K_1 and phytol were not blocked by methysergide (0·1 μM) or mepyramine (0·1 μM), but all were antagonized by atropine (0·1 μM). However, the responses to acetylcholine (1–100 nM) in hypoxia were not antagonized by vitamin K_3 suggesting that α-tocopherol, vitamin K_1 and phytol may be acting at a point before the muscarinic receptors in the colon and possibly involving a mechanism releasing acetylcholine.

This hypothesis was tested by using physostigmine (0·1 μM), which produced a marked potentiation of the responses to α-tocopherol, vitamin K_1 and phytol (Fig. 3). However, hexamethonium (1 μM), did not antagonize the responses to the stimulant agents and therefore

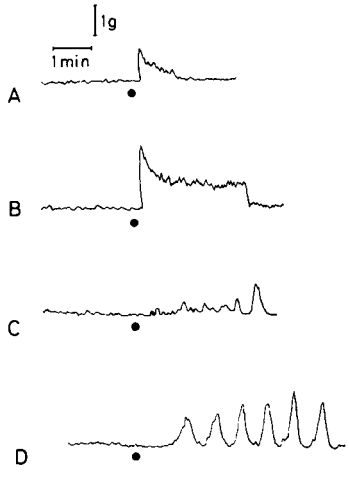

FIG. 3. Effect of physostigmine on responses to acetylcholine and α-tocopherol in guinea-pig isolated colon after hypoxic conditions had been established. Physostigmine was added 5 minutes before the other agents (●). Key: A acetylcholine (100 nM); B acetylcholine (100 nM) after physostigmine (0·1 μM); C α-tocopherol (20 μM); D α-tocopherol (20 μM) after physostigmine (0·1 μM).

this suggests that if the response is mediated through parasympathetic ganglia in the colon then it does not work through nicotinic receptors.

Previous attempts to analyse the mechanism of action of the components of the α-tocopherol molecule have revealed that the chromane ring structure contributes to an antioxidant effect and the phytyl side chain exhibits a membrane effect when measured on pulmonary vascular resistance and permeability (Seeger et al 1982). In addition, α-tocopherol binding sites have been demonstrated in various tissues (Catignani 1975; Kitabchi et al 1980).

The responses seen with α-tocopherol in hypoxic conditions occur within a concentration range equivalent to that found in normal human plasma, 20 μM (Hoppner et al 1970), and increase with increasing concentrations. This effect, because it is shown by physiological concentrations of α-tocopherol, could be a mechanism by which the body protects tissues from some of the consequences of hypoxia, possibly through stimulation of a 'vitamin E receptor' and because the effect is proportional to α-tocopherol concentration it might be amenable to dietary or pharmacological enhancement.

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