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VITAMIN E-SUPPLEMENTED DIETS REDUCE LIPID PEROXIDATION BUT DO NOT ALTER EITHER PITUITARY-ADRENAL, GLUCOSE, AND LACTATE RESPONSES TO IMMOBILIZATION STRESS OR GASTRIC ULCERATION

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It has been suggested that antioxidant administration to rats would reduce the physiological response to stress. In the present experiment adult male rats were given diets supplemented with vitamin E for one or seven days before they were subjected to immobilization stress. Vitamin E administration reduced hepatic and gastric lipid peroxidation in unstressed rats but did not modify the pituitary-adrenal, glucose and lactose responses to 1 or 18 h immobilization. Similarly, gastric ulceration caused by 18 h immobilization was unaffected by the diets. These results indicate that the inhibition of lipid peroxidation does not modify the response of several, well-known, stress-markers in the rat.

KEY WORDS: Stress, vitamin E, free radicals, lipid peroxidation, pituitary-adrenal axis, glucose, lactate, gastric ulceration.

INTRODUCTION

Free radicals have been implicated in a number of pathological processes caused by radiation, exposure to xenobiotic compounds, ischaemia and aging.[1-3] One of the most important consequences of exacerbated free radical formation is peroxidation of membrane lipids. Recently, it has been reported that some forms of stress such as exercise, starvation and even exposure to electric shock increase free radical concentration.[4-8] Furthermore, Meerson reported that both lipid peroxidation and some pathological changes associated with electric shock were prevented by administration of the antioxidant 2,6-di-tetrabutyl-4-methylphenol (ionol).[7] This author proposed that free radicals might be implicated in the control of general physiological response to stress.

However, no clear evidence for a protective role of vitamin E administration was found.[7] In addition, the same author has further reported that ionol administration inhibited catecholamine biosynthesis in the adrenals.[9] Therefore, the influence of free radical generation under stress on the physiological response to the stimuli are at present unclear. In the present experiment we assessed the effect of vitamin E administration in the diet on some physiological variables which are clearly altered by stress

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such as the pituitary-adrenal axis, the serum glucose and lactate levels, and gastric ulceration.[7,10,11]

MATERIALS AND METHODS

Male Sprague-Dawley rats approximately 60 days old were used. They were maintained in a controlled environment (lights on from 07.00 to 19.00, temperature 22°C) in groups of four per cage. Food and water were provided ad libitum. One week after their arrival the rats were randomly assigned to four experimental groups: 1) control rats that received normal diet (LETICA, Barcelona, Spain) containing 50 mg/kg vitamin E (α-tocopherol), 2) rats that received a supplement of vitamin E (0.8 g vitamin E/kg) in the diet for 7 days, 3) rats that received a diet supplemented with vitamin E (4 g/kg diet) just the day before they were subjected to stress, and 4) rats that received the latter diet for 7 days. After such treatments the rats were assigned to 3 different situations before sacrifice: a) no stress, b) 1 h immobilization, and c) 18 h immobilization. Those assigned to the latter group were stressed the day before in order to kill all animals at the same time. The rats were immobilized by attaching them to wood boards.[12] The animals were quickly decapitated in the morning. Trunk blood was collected and centrifuged at 4°. The serum was frozen at −20°. The stomachs were cut by the greater curvature, rinsed with saline and extended to measure the number and length of ulcers, which were restricted to the glandular portion of the stomach. Subsequently the stomachs were frozen at −90°C.

ACTH was determined by radioimmunoassay (RIA) using human I-ACTH (Du Pont NEN Res. Products, USA) as tracer, ACTH1-24 (Sigma, USA) as standard and rabbit human ACTH antiserum (hACTH22VO2). The antiserum was kindly provided by the NIDDK (Baltimore, Maryland, USA) through the National Hormone and Pituitary Programme. Corticosterone was determined by RIA using rabbit serum against corticosterone-3-OCMO (Bioclin, UK). No stress values were determined after previous extraction from serum with diethylether and stress values were determined without extraction as previously described.[13] Glucose and lactate were analyzed using commercially available kits from Boehringer Mannheim (Barcelona, Spain) and Biomerieux (Madrid, Spain), respectively. The stomachs were homogenized in 2 vol of ice-cold 10 mM TRIS-HCl pH 8.2, containing 0.25 M sucrose, 2 mM 2-mercaptoethanol, 10 mM sodium azide, and 0.1 mM phenylmethylsulphonyl fluoride and centrifuged at 50,000 g for 20 min at 4°C. The supernatants and homogenates were stored at −20°. Gastric lipid peroxidation was assessed by measuring malondialdehyde formation in the homogenates with the thiobarbituric acid method.[14]

All samples were processed in the same assay to avoid interassay variations. Two statistical comparisons were programmed: stress vs unstressed values with the Student t test and the influence of the diet under both unstressed and stressed conditions. In the latter case the vitamin E supplemented groups were compared to the control group with the Dunnett test ($\alpha = 0.05$).

RESULTS

The effect of vitamin E supplementation on the pituitary-adrenal, glucose and lactate responses to immobilization is depicted in Figure 1. The rats given the diet containing

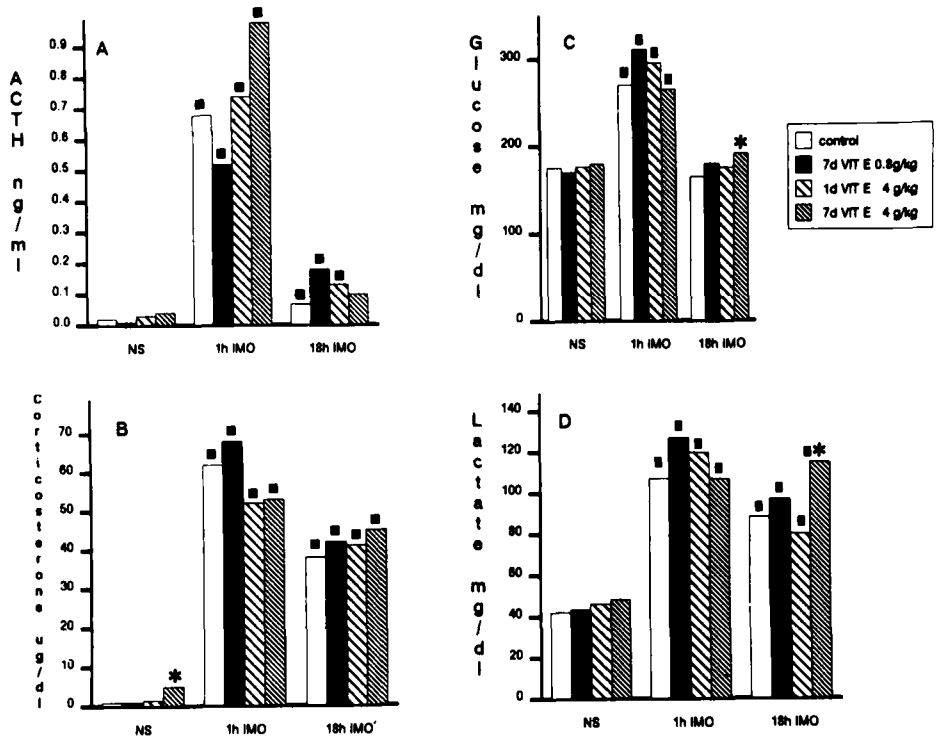

FIGURE 1 Effect of vitamin E supplementation on ACTH (panel A), corticosterone (panel B), glucose (panel C) and lactate (panel D) responses to stress. Means are represented. The rats were killed with no stress (NS, $n = 5$–6) or were subjected to 1 or 18 h immobilization (IMO, $n = 7$–10). ■ $p < 0.05$ vs the corresponding nonstressed group, *$p < 0.05$ vs the control group under the same acute treatment.

the highest supplement of vitamin E for 7 days showed higher basal serum corticosterone levels than control rats but normal ACTH levels. Both ACTH and corticosterone responses to immobilization were similar in all animals irrespective of vitamin E content of their diets. Basal serum glucose and lactate concentrations were unchanged by the diets, as was the response to 1 h immobilization. However, the rats given the diet containing a supplement of 4 g/kg vitamin E showed higher serum glucose and lactate levels than control rats after 18 h immobilization.

Stomach ulceration after 18 h immobilization was similar in all experimental groups (Table 1). The effectiveness of vitamin E treatments was supported by their effects on gastric lipid peroxidation (Table 1). Both acute and chronic vitamin E supplementation (4 g/kg) significantly reduced TBA-reactive products in the stomach. However, immobilization stress did not modify gastric lipid peroxidation in any group.

DISCUSSION

The present results indicate that vitamin E-supplemented diets effectively reduced the amount of TBA-reactive products in the stomach. We have previously reported

TABLE 1

The effect of vitamin E supplementation on gastric lipid peroxidation (LPO) and the degree of gastric ulceration in 18 h immobilized rats

Treatment	Gastric LPO μmol/g		Gastric ulcers	
	Unstressed	Stressed	Number	Total length (mm)
Control	110.7 ± 11.6 (6)	91.2 ± 9.9 (10)	1.9 ± 0.5 (10)	3.9 ± 2.2
Vit Ec1	-	-	2.3 ± 0.5 (9)	4.6 ± 1.9
Vit Ea2	65.3 ± 6.5* (5)	62.2 ± 7.6* (9)	2.2 ± 0.5 (9)	4.4 ± 1.4
Vit Ec2	54.3 ± 6.4* (5)	42.7 ± 3.0* (5)	1.0 ± 0.4 (9)	2.3 ± 0.8

Means ± S.E.M. are represented. The number of rats per group is in parentheses. The different groups are identified as follows: vit Ec1 indicates rats given a diet supplemented with 0.8 g vitamin E/kg for 7 days, Ea2 and Ec2 indicate rats given a diet supplemented with 4 g vitamin E/kg for 1 or 7 days, respectively.
*$P < 0.05$ vs the corresponding control group.

similar results in the liver of the same animals.[6] This supports the hypothesis that vitamin E is an effective antioxidant in the rats.[2] It has been reported that administration of the antioxidant ionol to rats subjected to 6 h of emotional painful stress, significantly reduced not only lipid peroxidation in the heart and the brain but stomach ulceration and corticosterone response as well.[7] These results were interpreted as supporting the hypothesis that ionol administration modified the control systems of the organisms, inhibiting the general response to stress.[7]

However, our results demonstrate that diminishing lipid peroxidation by means of vitamin E administration does not reduce any of the various dependent variables related to the response to stress: pituitary-adrenal axis, glycaemia, lacticacidaemia and gastric ulceration. With regard to the pituitary-adrenal axis, it is noteworthy that chronic vitamin E administration increased basal serum corticosterone without altering ACTH concentration. This might have been due to the positive effect of vitamin E on steroid biosynthesis in the adrenal.[15,16]

Tariq[17] reported that oral vitamin E administration 30 min before the exposure to stress or the administration of various necrotizing agents protected gastric mucosa. The amount of vitamin E administered was very similar to that ingested by the rats in the present experiment (diet of 4 g vitamin E/kg). We have repeated the experiment and no protection from gastric ulceration caused by 2 h immobilization at 4° was found (data not shown). The discrepancies might have been due to the fact that the above-mentioned authors administered vitamin E 30 min before the exposure stress (2 h restraint at 3°). Instead, we used 18 h immobilization at 22°, with no access to food and water. Therefore, it seems likely that the amount of vitamin E in the gastrointestinal tract during stress was much higher in Tariq's experiment than in ours. In any case, our data indicate that administration of vitamin E-supplemented diets which partially inhibited gastric (present data) and hepatic[6] lipid peroxidation was not sufficient to reduce gastric ulceration caused by stress. In addition, 18 h immobilization increased lipid peroxidation in the liver[6] but not in the stomach. Apparently, therefore, gastric ulceration caused by stress is not causally related to any increase in lipid peroxidation, not does a significant decrease in lipid peroxidation significantly protect gastric mucosa. The results obtained by Tariq[17] might be due to a more pronounced decrease in lipid peroxidation or to other effects of high levels of vitamin E in the stomach.

It seems likely that ionol inhibited some of the responses to stress by inhibiting

catecholamine biosynthesis. Thus, Meerson *et al.* reported that administration of the β-antagonist inderal prevented most of the pathology associated with stress.[7] Secondly, it has recently been demonstrated that ionol blocks catecholamine biosynthesis in the adrenal medulla by inhibition of dopamine-β-hydroxylase.[9] A similar action might take place within the central nervous system. Since catecholamines are implicated in the induction of gastric ulceration[18] and in the control of the pituitary-adrenal axis[19] during stress, ionol might actually inhibit both responses by its action on catecholamine biosynthesis. It is unlikely that vitamin E administration exerted any major effect on catecholamine secretion during stress because hyperglycaemia was similar in control and vitamin E-treated rats and no effect of vitamin E administration on catecholamine release during stress has been observed in pigs.[20]

In conclusion, the present results suggest that vitamin E supplementation of the diet, although partially inhibiting lipid peroxidation, does not diminish the intensity of the physiological response to a strong stressor such as immobilization.

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