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Lack of Effect of α-Tocopherol and Ascorbic Acid on the Response of Some Physiological Variables to Immobilization Stress in Rat

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The effect of α-tocopherol or ascorbic acid jointly and separately on the seric levels of adrenocorticotropin (ACTH) and corticosterone has been studied. ACTH response to stress is similar in all groups, whereas the corticosterone response is higher in the animals treated with α-tocopherol. Neither lactate response nor gastric ulceration caused by stress was influenced by the administration of α-tocopherol and/or ascorbic acid. These results suggest that free radicals might not be implicated in the control of the physiological response to stress.

Key words: Ascorbic acid, Tocopherol, Pituitary-adrenal axis, Stress, Antioxidants.

Free radicals are those atoms or molecules having one or more unpaired electrons in an outer orbital. Due to the presence of unpaired electrons these compounds have high reactivity. It is now well established that aerobic organisms produce free radicals as a consequence of their metabolic processes. Superoxide (O^{2-}) and hydroxyl (OH) are the most relevant free radical species in the organisms, the hydroxy radicals being exceedingly active. Fatty acids components of membrane lipids are specially sensitive to hydroxyl radicals and they are transformed in lipid peroxides with the subsequent disruption of some membrane-associated processes (11, 14). Free radicals have been implicated in a number of physiological and pathological processes related to inflammation, ischemia, aging, radiation and inhalation or ingestion of xenobiotic compounds (5, 8).

The organisms have enzymatic and non enzymatic defenses against free radicals. Of the non enzymatic defense α-tocopherol appears to be the better hydrophobic antioxidant (5). Ascorbic acid has been also considered a hydrophylic antioxidant (2). In addition, a potentiation by ascorbic acid of the effects of α-tocopherol appear to exist *in vivo* (2).

It has been reported that different

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stressful situations increase lipid peroxidation in various organs (3, 4, 9, 11, 13, 16). Administration of a liposoluble antioxidant (ionol) reduced lipid peroxidation in the heart and the brain of rats and also corticosterone response and gastric ulceration (11). The author proposed that administration of antioxidant might block the general response to stress. The aims of the present experiment were to investigate: a) the influence of natural antioxidants on the physiological response to immobilization stress, and b) the possible existence of a synergistic effect between α-tocopherol and ascorbic acid.

Materials and Methods

Male Sprague-Dawley rats approximately two months old were used. They were maintained in a controlled environment (lights on 7-19 h, temperature 22 °C) for at least one week before starting the experiment. Food and water were provided *ad libitum*. The rats were assigned to four groups and treated all of them for four consecutive days: a) rats injected i.p. with saline and s.c. with olive oil in the morning (controls); b) rats receiving s.c. α-tocopherol solved in olive oil (100 mg/kg); c) rats receiving i.p. ascorbic acid (500 mg/kg) and d) rats receiving the two substances at the doses indicated above. All rats were injected with the same vehicles as the other rats. α-Tocopherol and ascorbic acid were obtained from Roche (Spain).

After the last injection several rats from each group (n = 5) were left undisturbed. The others were subjected to food, but not water deprivation until the next day. Then, the latter animals were immobilized for 2 h in a cold room (4 °C) by attaching them to wood boards. Unstressed and stressed rats were killed by decapitation in an area adjacent to both the animal room and stress room. Trunk blood was collected and centrifuged at 4° C. 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.

Serum ACTH was determined by radioimmunoassay (RIA) using human ^{125}I-ACTH (Du Pont NEN Res. Products, USA) as tracer, ACTH1-24 (Sigma) as standard and rabbit antiserum against human ACTH (hACTH22VO2) provided by the NIDDK. Serum corticosterone was determined by RIA using rabbit serum against corticosterone-3-OCMO (Bioclin, UK). Non stress values were determined after previous extraction from serum with dyethylether and stress values were determined without extraction as previously described (1). Serum lactate was analyzed using a commercially available kit (Biomerieux).

All samples were processed in the same assay to avoid interassay variations. Two statistical comparisons were programmed: a) stressed versus unstressed values with the Student t-test or the Mann-Whitney U-test (gastric ulceration), and b) one-way ANOVA of the four experimental groups under the same acute (stress, no stress) treatments. Where appropriate, post-hoc comparisons between means were carried out with the Duncan test.

Results

The effect of α-tocopherol and/or ascorbic acid administration on basal and stress levels of ACTH, corticosterone and lactate in serum is depicted in table I. The basal levels of these variables were not modified by the administration of α-tocopherol and ascorbic acid. The response of the three variables to 2 h immobilization was highly significant in all cases. Of the variables analyzed, only a significant effect of α-tocopherol administration on corticosterone response to stress was found. Neither the number of ulcers

Table I. *Effect of α-tocopherol (T) and ascorbic acid (A) administration on ACTH, corticosterone and lactate responses to 2 h immobilitation at 4 °C.*
Means ± SEM are represented. The number of animals per group is in parentheses. * $p < 0.05$ vs its corresponding non stressed group, # $p < 0.05$ vs the control group under stress.

	Control	n	α-tocopherol	n	Ascorbic ac.	n	T + A	n
ACTH (pg/ml)								
No stress	14.0 ± 2.5	(5)	29.2 ± 8.8	(5)	16.0 ± 2.5	(5)	15.0 ± 2.3	(5)
Stress	267.7 ± 51*	(9)	311.2 ± 46*	(8)	233.1 ± 33*	(8)	228.7 ± 18*	(8)
Corticosterone (μg/dl)								
No stress	1.2 ± 0.5	(5)	1.9 ± 0.9	(4)	4.0 ± 1.2	(5)	1.2 ± 0.5	(4)
Stress	61.8 ± 3.6*	(9)	82.2 ± 7.9*#	(9)	75.9 ± 8.1*	(9)	71.6 ± 4.7*	(9)
Lactate (mg/dl)								
No stress	69.3 ± 2.9	(5)	73.4 ± 3.9	(5)	76.6 ± 2.7	(5)	76.1 ± 1.1	(5)
Stress	110.0 ± 5.3*	(9)	115.9 ± 5.4*	(9)	113.3 ± 3.8*	(9)	120.0 ± 8.4*	(9)

Table II. *Effect of α-tocopherol (T) and ascorbic acid (A) administration on gastric ulceration caused by immobilization (2 h at 4 °C).*
Means ± SEM (n = 9) are represented.

Treatment	Number of ulcers	Cumulative length (mm)
Control	8.4±1.9	13.6±3.3
α-Tocopherol	9.8±2.3	19.2±5.4
Acorbic acid	7.9±2.1	19.9±5.0
T+A	7.8±1.2	17.6±2.6

caused by immobilization nor their cumulative length were significantly altered by α-tocopherol and/or ascorbic acid administration (table II).

Discussion

It was found that neither α-tocopherol nor ascorbic acid administration altered the ACTH response to stress in rats. Similarly, serum lactate levels and the degree of gastric ulceration caused by immobilization were similar in all groups. These results argue against the hypothesis that the administration of antioxidants would prevent the physiological response to stress (11). This hypothesis assumes that some physiological signal triggers by free radicals would reach the brain centers controlling the physiological response to stress. This was based on the finding that administration of the antioxidant ionol significantly reduced stress-induced lipid peroxidation in the brain and the heart, plasma corticosterone release and gastric ulceration (11). However, some of the effects of ionol on the above mentioned variables could be explained by its inhibitory action on catecholamines biosynthesis (12) rather than by its antioxidant properties.

The lack of protective effect of ascorbic acid on gastric ulceration caused by stress is in accordance with several reports (6, 7, 10). In fact, a potentiation of gastric ulceration caused by either stress or aspirin administration has been observed (6, 7, 10). α-Tocopherol administration has been reported to reduce gastric ulceration (15). The discrepancies between our results ant those of Tariq could be due to the time elapsed between the last α-tocopherol administration and the beginning of exposure to stress (approximately one day in our experiment and 30 min in the Tariq's experiment). Probably a critical concentration of α-tocopherol in either the stomach or the blood is needed to protect gastric mucosa.

In sum, the present results suggest that

neither ascorbic acid nor α-tocopherol administration, nor a combination of both significantly exerted any major effect on the physiological response to stress evaluated by the pituitary-adrenal and lactate responses and the degree of gastric ulceration.

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Resumen

Se estudia en ratas machos el efecto del α-tocoferol o del ácido ascórbico conjuntamente y por separado, sobre los niveles séricos de adrenocorticotropin (ACTH) y corticosterona. La respuesta de la ACTH al estrés es similar en todos los grupos, en tanto que la respuesta de la corticosterona es mayor en los animales tratados con α-tocoferol. Ni los niveles de lactato, ni el grado de ulceración gástrica causados por el estrés se alteran por la administración de α-tocoferol o ácido ascórbico. Estos datos sugieren que el grado de formación de radicales libres durante el estrés no modifica la respuesta fisiológica al mismo, en la rata.

Palabras clave: Acido ascórbico, Tocoferol, Eje pituitario adrenal, Estrés, Antioxidantes.

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