EFFECT OF α-TOCOPHEROL ON CONDITIONED-REFLEX ACTIVITY IN ADRENALIN-INDUCED MYOCARDIAL DYSTROPHY IN RATS

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UDC 616.127-007.17-02:615.357:577.175.522]-092:
[612.833.81.014.46:615.356.577.161.3

KEY WORDS: adrenalin-induced stress; myocardial dystrophy; antioxidant; conditioned reflex; afferent synthesis; amnesia.

Adrenalin induces a stress reaction which is expressed as a twofold increase in the blood corticosteroid level and a tenfold increase in the adrenalin and noradrenalin levels in the heart [6]. As early as 1 h after intramuscular injection of adrenalin into rats in a dose of 2 mg/kg areas of microfocal dystrophy appear in their hearts, followed 2 h later by massive thrombosis of the coronary vessels [5, 12]. Later necrobiotic changes in the cardiomyocytes [5], ECG disturbances, and death of some of the experimental animals supervene [7]. The levels of lipid peroxidation (LPO) products, namely diene conjugates and malonic dialdehyde, in the myocardium of rats receiving adrenalin later rise 2 and 5 times respectively [10]. Despite such damage to the heart and other internal organs in stress, acceleration of formation of conditioned reflexes may be accelerated and their preservation enhanced, so that the brain is able to perform its basic function of controlling behavioral responses of the animal more intensively.

The aim of this investigation was to test the hypothesis [11] of selective protection of the brain by studying the degree of preservation of conditioned reflexes in adrenalin myocardium [10] or adrenalin-induced myocardial dystrophy [4-7, 10, 12] with or without the prophylactic use of an antioxidant.

EXPERIMENTAL METHOD

Experiments were conducted on 50 noninbred male albino rats weighing 250-280 g. Adrenalin-induced myocardial dystrophy (AIMD) was created by a single intramuscular injection of adrenalin in a dose of 2 mg/kg, known to give rise to necrosis [14]. Before reduction of AIMD, the unilateral delayed conditioned reflex was formed in all (50 rats). The conditioned stimulus was photic, the unconditioned electrical (36 V). Delay was reduced by reinforcing the conditioned stimulus at the 2nd minute of its action. However, during realization of the conditioned reflex with a short latent period (LP) we reinforced it without waiting 2 min. Delay of the conditioned active avoidance reflex (CAAR) thus gradually shortened. The CAAR was formed [14] by the use of the chamber usually used to form a conditioned passive avoidance reflex (CPAR), the rat being placed immediately in the dark and dangerous (and with electrically conducting floor) compartment and the door was closed for 60 sec. The light (conditioned stimulus) was then switched on and the door opened. At the 2nd minute of action of the conditioned stimulus, it was reinforced by electrical stimulation (36 V). This caused an unconditioned avoidance reflex in the rats. The combination of photic and electrical stimulation repeated 10-15 times led to fixation of CAAR. To achieve required criterion of training (up to 68-84%) 3 to 5 experimental days were needed (five combinations per day). After firm consolidation of the conditioned reflex, AIMD was induced, by injection of adrenalin into all rats of the five groups (50 rats). The state of the rats' conditioned-reflex activity was tested 1, 3, 24, 48, and 72 h after induction of AIMD. Each group of experimental rats, including the control group with CAAR, consisted of 10 rats. Group I consisted of rats with pure AIMD and tested in Frunze (760 m above sea level), group II consisted of rats with a developed CAAR stereotype and receiving α-tocopherol in a dose of 75 mg/200 g daily for 1 week before induction of AIMD in Frunze, group III of rats with CAAR and receiving α-tocopherol with

Laboratory of Pathophysiology, Institute of Physiology and Experimental Pathology of High Altitudes and Laboratory of Pharmacology, Institute of Organic Chemistry, Academy of Sciences of the Kirghiz SSR, Frunze. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 3, pp. 211-213, March, 1990. Original article submitted August 17, 1988.

0007-4888/90/0003-0263$12.50

TABLE 1. Changes in LP of CAAR during AIMD with and without Prophylactic Administration of α-Tocopherol in Rats ($M \pm m$)

Experimental conditions	LP before and after development of AIMD, sec						Number of surviving rats, in % of total number of animals
	initially	after 1 h	after 3 h	after 24 h	after 48 h	after 72 h	
AIMD (control)	8,9±1,6	14,6±2,4*	15,5±2,6	16,6±3,4	28±4,2	7,8±1,8	50,0
p	<0,01	<0,001	<0,001	<0,05	<0,001	>0,5	
AIMD + prophylactic injections of α-tocopherol for 1 week	6,5±1,2	4±0,9	10,9±0,8	10,9±0,8	9±0,7	9,3±1,8	88,8
p		<0,01	<0,001	<0,05	<0,01	<0,01	
AIMD + prophylactic injections of α-tocopherol for 1 day	5,4±1,5	10,9±2,4	8,8±1,4	12,5±2,4	10,7±1,6	14,2±1,2	90,0
p		<0,05	<0,1	<0,01	<0,01	<0,001	
AIMD + prophylactic injections of α-tocopherol for 1 week	3,9±1,2**	4,1±0,8	6,8±1,2	5,9±0,8	4,7±0,8	5±1,2	100,0
p		>0,5	<0,05	<0,05	>0,5	>0,5	
AIMD preceded by adaptation at an altitude of 1600 m and propylactic α-tocopherol for 1 day	9,6±1,4	9±1,8	5,6±0,8	8,6±1,3	11,2±0,9	13,3±11,8	100,0
p			<0,05	<0,5	<0,1	<0,01	

Legend. *) Comparison with initial value of LP, and so on; **) α-tocopherol injected on days of CAAR formation, by contrast with animals of group II, receiving the antioxidant after formation of the CAAR stereotype, although in both cases prophylactic administration of the antioxidant for 1 week preceded AIMD.

a single dose 24 h before induction of AIMD in Frunze, group IV of rats receiving α-tocopherol, by contrast with the rats of group II, on the days of CAAR formation, for 1 week. Later AIMD was also induced in these rats in Frunze. Group V consisted of rats receiving a single dose of α-tocopherol after CAAR production and before AIMD induction, against the background of adaptation in the mountains (1600 m above sea level, on the shore of lake Issyk-Kul') after CAAR formation and before induction of AIMD. The duration of LP (in sec) of the conditioned reflex was taken as the rate of realization of afferent synthesis [13]. The dose of α-tocopherol was 75 mg/200 g body weight, either singly or daily for 1 week.

EXPERIMENTAL RESULTS

Injection of adrenalin alone caused changes in LP of CAAR in the surviving rats (50% of cases) of group I. A threefold ($p < 0.001$) increase in LP was observed between 1 and 48 h after induction of AIMD, and the normal value was not restored until the 3rd day (Table 1). The number of CAAR realized was increased to 92% (compared with the initial value of 68%).

As Table 1 shows, LP in rats protected against AIMD with α-tocopherol (group II) was shortened in the early period of heart damage (after 1 h), but lengthened after between 48 and 72 h (to 9-10.9 sec) compared with its initial value (6.5 sec). The positive effect of the antioxidant was also expressed as maintenance of the initial level of conditioned reflexes realized (68 and 71.4%). The better state of afferent synthesis (to judge by the short duration of LP) and the sharp decline in mortality (11.2% compared with 50% in the control) are evidence of the prophylactic and cerebroprotective effect of the natural antioxidant. It was noted above that the second type of prophylactic administration of the antioxidant consisted of injection of a single dose before AIMD (group III). For 3 days LP was moderately lengthened and after 72 h of AIMD development it reached 14.2 ± 1.2 sec (compared with 5.4 sec initially). Between 24 h and 72 h of AIMD development the incidence of amnesia was increased by 2.5 times in the surviving (90%) rats of this group (Table 1).

In the rats of group IV, LP remained within normal limits (4.1-6.8 sec) during 3 days of AIMD, and the incidence of amnesia was lowest (from 14 to 24). The survival rate in this group of experimental rats was 100%, evidence of the effectiveness of this type (daily injections of α-tocopherol coinciding with CAAR formation during 1 week before AIMD) of antioxidant prophylaxis.

The last group (V) of investigations was conducted in the mountains on animals adapted to an altitude of 1600 m for 15 days. Before AIMD α-tocopherol was injected only once. Their LP between 3 and 72 h after the development of AIMD varied within normal limits (3.3-5.6 compared with 9.6 initially), and the survival rate of the rats was 100% (Table 1).

AIMD is thus accompanied, first, by death of the experimental animals (according to our data, sudden death took place in 50% of the control animals, 16% below values reported by other workers) [10]. Second, afferent synthesis, reflected in lengthening of LP, worsened in the surviving rats (50%). Prophylaxis of AIMD with the antioxidant considerably reduced mortal-

ity (by 40%). In some types of α-tocopherol prophylaxis of AIMD, 100% of animals survived. The antioxidant also significantly corrects the character of the change in conditioned-reflex activity; lengthening of LP is moderate in character and realization of conditioned reflexes remained at the background level (in %). Antioxidant prophylaxis preceded the simultaneous action of the two stress-inducing factors (learning based on nociceptive reinforcement and AIMD), the animals not only survived in 100% of cases, but they also preserved their initial pre-stress level of afferent synthesis, with maximal realization of CAAR.

What is the mechanism of the cardio- and cerebroprotective action of α-tocopherol? It has been shown that free radicals damage the myocardium, and that α-tocopherol has a protective action [15], manifested in particular as lowering the level of induced chemiluminescence [3]. In the case of prevention of oxidation by α-tocopherol, it activates enzymic and nonenzymic scavenging of superoxide radicals in the brain [8]. By enhancing antiradical protection of the brain α-tocopherol also optimizes conditioned-reflex activity, and LP of conditioned reflexes is shortened [9], i.e., afferent synthesis is improved [13]. A similar fact was discovered in our experiments with prevention of AIMD by α-tocopherol. It was discovered at the same time that high-altitude adaptation and α-tocopherol exert an additive cardio- and cerebroprotective effect in stress. For example, the 100% survival of the mountain rats with AIMD was accompanied by preservation of the initial level of LP of the conditioned reflexes of these rats, although cases of retrograde amnesia were observed.

Thus the effect observed after a single injection of the antioxidant before injection of stress- and necrosis-inducing doses of adrenalin, calls for special explanation. In our view, adaptation in the mountains, the molecular mechanism of which consists of enhancing the antioxidant activity of the blood plasma lipids and reducing the degree of malonic dialdehyde generation in the platelets [1, 2], reduces the cardio- and cerebroprotective dose of α-tocopherol in stress. Under ordinary lowland conditions, only prophylactic administration of the antioxidant for 1 week can guarantee 100% survival and preservation of afferent synthesis, accompanied by better realization of conditioned reflexes, in animals with heart damage (AIMD). The antioxidant also increases the resistance of the brain and preserves a high level of conditioned-reflex activity.

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