EFFECT OF THE CALCIUM ANTAGONIST VERAPAMIL ON SUPEROXIDE DISMUTASE ACTIVITY AND CONDITIONED-REFLEX MEMORY IN RATS WITH ADRENERGIC CARDIONECROSOGENIC STRESS

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In any form of stress accompanied by stimulation of lipid peroxidation (LPO) various types of changes arise in neuron membranes. Under normal conditions LPO is one of the mechanisms regulating the state of membranes and it is controlled in all its stages by the antioxidant system; superoxide dismutase (SOD) inhibits LPO [6, 7]. The opinion has been expressed that excess of Ca^{2+} in stress causes not only hyperactivation of the neuron, but also intracellular injury, although as regards the degree of antiradical protection, the brain occupies a "privileged" position [8]. In their opinion, exposure to stress induces dissimilar levels of formation of LPO products in the brain, heart, and other organs. There is information showing that in stress SOD activity rises rapidly in the brain, and in emergency it abolishes excessive activation of LPO [2]. Activation of LPO and an excess of Ca^{2+}, by injuring neurons, may lead to their death, i.e., to "calcium death" [7]. Ca^{2+} antagonists protect neurons still undamaged or reversibly damaged against death. Verapamil regulates calcium inflow into neurons and is an effective corrector of their activity and vulnerability [4]. The Ca^{2+} level regulates "cell memory."

The aim of this investigation was to study the effect of the calcium antagonist verapamil on SOD activity and conditioned-reflex memory.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male and female albino rats (9-10 animals in each group). Adrenergic cardionecrosogenic stress (ACNS) was induced by adrenalin in a dose of 2 mg/kg [3]. To study conditioned-reflex memory, the conditioned passive avoidance reflex method (PCAR) was used, in a special chamber consisting of two compartments: light and dark. The rat was placed in the light half of the chamber with its tail toward the door. During the formation of an unconditioned "burrowing reflex" [1] and entry of the rat into the dark half of the chamber, it immediately received nociceptive electrodermal stimulation (EDS, 0.75 mA, 2 sec). After the first training period (avoidance) the rat was transferred into a common cage. The time of observation was 180 sec. The CPAR thus formed was tested after 1, 2, 3, 6, and more days. Verapamil was injected intraperitoneally in a dose of 12.5 mg/kg. Superoxide dismutase in brain and heart homogenates was determined by the method in [9, 10] in 10 rats 24 h after ACNS, and specially selected for this series of investigations. CPAR was studied in the main series (also 10 rats) after they had developed ACNS. Conjugated dienes (CD) were determined as in [13].

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TABLE 1. Change in Superoxide Dismutase Activity of Brain and Heart without and against the Background of Verapamil, in ACNS (M ± m, n = 10)

Series	Conjugated dienes, nmoles/mg lipids		SOD activity, units		Ratios	
	brain	heart	brain	heart	brain CD/ heart CD	brain SOD/ heart SOD
Control (intact rats)	0,39±0,04	0,40±0,03	593±22	415±31	0,9	1,4
ACNS	0,31±0,03	0,57±0,03*	521±16*	345±15*	0,5	1,5
Verapamil + ACNS	0,35±0,04	0,35±0,03	526±14*	328±5*	1,0	1,6

Legend. Here and in Table 2, values for which $p < 0.05$ compared with control marked by an asterisk.

TABLE 2. Characteristics of Fixation and Consolidation of CPAR under Normal Conditions and in Stress with and without Verapamil (M ± m, n = 9-10)

Series	Latent outcome, % of total number	Fixation and consolidation of CPAR, % after undermentioned no. of days				LP of passage from light into dark compartment, sec after undermentioned number of days			
		1	2	3	4	1	2	3	4
Control	0	70,0	70,0	100,0	100,0	128,9±14,5	128,9±14,5	0	0
ACNS	22,2	30,0	60;0	76,6	100,0	95,5±10,0*	96,6±8,5*	129,6±12,0	0
Verapamil + ACNS	0	50,0	70,0	80,0	100	87,7±17,2*	128,8±9,6	125,1±13,2	0

Legend. O) Absence of passage from light into dark compartment during period of observation (180 sec), i.e., stable CPAR.

EXPERIMENTAL RESULTS

As Table 1 shows, 24 h after ACNS the content of primary LPO products (CD) in the heart was increased by 29.8% ($p < 0.001$). This was not observed, however, in the brain. Verapamil completely prevented the increase in CD in the heart after ACNS. In the brain, however, irrespective of prevention of stress by verapamil, the CD level was not increased. As regards antiradical protection of the brain, judging by its SOD activity, it was moderately depressed in ACNS (by 12.1%). Verapamil did not prevent even this moderate reduction of SOD activity of the brain during stress. The onset of sudden death (SD) 24 h after ACNS was observed in 22.2% of cases. However, there was 100% survival of rats receiving verapamil. Calculation of the brain CD/heart CD ratio showed that in ACNS it was reduced almost by half, but against the background of verapamil it remained within normal limits (1.0 and 0.9). Verapamil, as a blocker of slow Ca channels, maintains the relatively "privileged" position of the brain to some extent during stress; it inhibits the reduction of brain SOD activity moderately.

Calculation of the brain SOD/heart SOD ratio showed that in adrenergic stress homeostasis of SOD between the two target organs remains stable, but verapamil increases it (to 1.6 from the normal value of 1.4). This is a sign of protection of the brain. All this plays an important role in the formation of CPAR and its preservation in rats exposed to stress with cardiac damage (ACNS).

As Table 2 shows, adrenergic cardionecrosogenic stress, accompanied by intensification of LPO in the heart, impairs fixation of the CPAR (30% compared with 70%). 100% fixation of CPAR in these animals occurred on the 4th day, i.e., 1 day later than in intact rats. The slight inhibition of brain SOD activity during prevention of ACNS by verapamil played an essential role, and early fixation of CPAR (after 24 h) differed quantitatively; in prevention of ACNS by verapamil fixation of the conditioned reflex was significantly greater than without it (50% compared with 30%; with a delay of 1 day, moreover).

An epoch equal to 180 sec in the intact rats was observed for 3 days, but in the stressed rats (ACNS) on the 4th day, just as with the rats receiving verapamil.

Physiological correlation was thus bound between the reduction of antiradical protection (SOD) of the brain and fixation of the CPAR in it during adrenalin-induced stress with damage to the heart. The brain is better protected against LPO products than the heart [8], although ACNS initiated a moderate decrease in SOD in the brain. This evidently served as the basis for delay in formation of conditioned reflex memory (CPAR), which was expressed as frequent passage of the rats into the punishable compartment of the chamber for a comparatively long time (4 days instead of 3 days in the control). This type of pathological processes in the brain [6] is transient in character, just like the process of stress-induced (adrenergic) injury to the heart [3].

The Ca^{2+} antagonist verapamil, if given during lethal adrenergic stress, not only protects the heart and brain, for it led to survival of the "adrenalin" in rats from calcium overloading and "calcium death" in 100% of cases. The "verapamil" and control rats restored their ability to fix (in 100% of cases) conditioned-reflex memory (CPAR) with a delay of 1 day. Weak activity of verapamil in modulation of conditioned-reflex integrative activity of the brain and activity of SOD during stress can evidently be explained on the grounds that, as a rule, the Ca channels of the brain membranes are less sensitive to calcium antagonists than Ca channels in the myocardium [12]. Verapamil blocks only open channels and does not block Ca-channels in any state. Verapamil is characterized by a paradoxical response of neuromuscular synapses: an increase in spontaneous secretion of mediator and disinhibition of its secretion. The authors cited link this effect with an increase in Ca^{2+} release from the intracellular storage depots. The 100% effect on survival of rats with lethal adrenergic stress against the background of verapamil prophylaxis is therefore connected, we consider, mainly with abolition of the "calcium overloading" of the heart. Without verapamil 22.2% of the animals died, and in those which survived, belonging both to "purely adrenalin" and "verapamil" groups, fixation and consolidation of CPAR were about eaual in character, i.e., the brain is relatively resistant to this type of stress, even without prevention by the calcium antagonist. Our results agree with those in [11], which showed that verapamil, in a dose of 0.6 mg/kg (12.5 mg/kg in our experiments) prevented ventricular fibrillation of the heart in 100% of guinea pigs. The authors cited associated the antiarrhythmic and antifibrillatory effect of the Ca^{2+} antagonist with the blocking of Ca^{2+} release into cardiomyocytes in myocardial ischemia.

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