Effect of Citrocard on Functional Reserves of the Heart under Conditions of Chronic Stress

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Chronic stress exposure produces a damaging effect on the myocardium and reduces its functional (inotropic) reserves. Citrocard (50 mg/kg) and fenibut (50 mg/kg) prevent stress effects: animals receiving these preparations demonstrate higher contraction and relaxation rates and higher left-ventricular pressure during functional tests (volume load and maximum isometric load).

Key Words: *chronic stress exposure; volume load; maximum isometric load; parameters of myocardial contractility*

Stress exposure leads to excitation of the hypothalamic-pituitary-adrenal system resulting in increased catecholamine concentrations, vasoconstriction, hypoxia, and intensification of LPO followed by destruction of cell membranes and impairment of energy supply, microcirculatory disturbances, structural changes in cardiomyocytes, depression of the contractile function of the heart and impairment of its functional reserves [2,5].

Hyperexcitation of the hypothalamic-pituitary-adrenal system and disturbances in heart structure and metabolism can be prevented by activation of the stress-limiting systems, *e.g.* GABAergic system [2,5]. The search and testing of substances possessing GABA-positive effects as potential cardioprotectors in stress-induced damage to the heart are an actual problem.

Here we studied the effects of GABA derivatives citrocard and fenibut (reference preparation) on functional reserves of the heart under conditions of chronic stress exposure.

MATERIALS AND METHODS

Experiments were carried out on 24 male Wistar rats weighing 280-320 g. The animals were divided into 4 groups (6 rats per group). Group 1 comprised intact animals (positive control), group 2 (negative control) included rats daily receiving intraperitoneal injections of physiological saline 60 min before stress exposure (over 2 weeks), groups 3 and 4 consisted of rats receiving citrocard (50 mg/kg) or fenibut (50 mg/kg), respectively, according to the same scheme.

A model of 2-week inescapable stress including immobilization and painful electrical stimulation was used in the experiment. The animals were placed in boxes (20×10×10 cm) of an experimental setup; electric current was delivered to the electrode floor. Every day, 60 stimulation sessions were performed over 1 h (15-sec stimulation and 45-sec interval); the initial voltage was 20 V, the voltage increased by 10 V after presentation the 20th and 40th stimuli [7]. The animals were kept in individual cages throughout the stress period.

For evaluation of the parameters of myocardial contractility, the animals were narcotized with sodium etaminal (40 mg/kg), artificial ventilation was started, the thorax and then the pericardium were opened, and a catheter was introduced through the apex into the left ventricle. Left-ventricular pressure and its first derivative (dp/dt+ and dp/dt-) were recorded using a hemodynamic analyzer equipped with BEAT software. After recording the initial

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parameters, functional tests were performed in the following order: volume load (intravenous bolus injection of 3 ml/kg physiological saline) and maximum isometric load (30-sec clamping the ascending aorta). The intensity of structure functioning and maximum intensity of structure functioning (MISF) were calculated by the formula: (LFPm× HRm)/(weight of left ventricle+$^1/_3$ interventricular septum).

The data obtained in the control and experimental groups were compared. If the parameters of myocardial contractility in groups of stressed animals receiving the test preparations surpassed the corresponding values in the negative control, a conclusion was made on cardioprotective effects of these preparations.

The data were processed by Newman—Keuls test ($M\pm S$) using Statistica software.

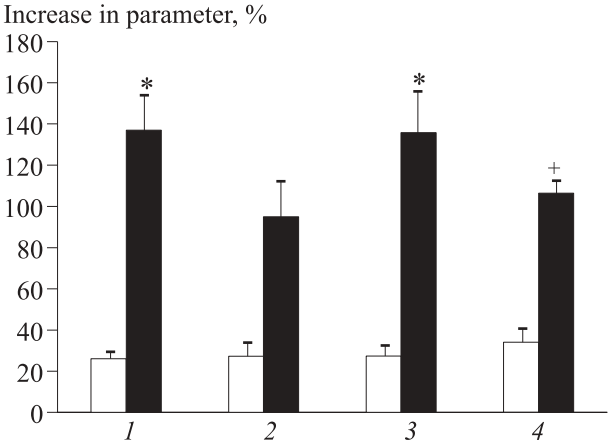

Fig. 1. Changes in intensity of structure functioning (light bars) and MISF (dark bars) under conditions of chronic stress. *p<0.05 compared to: *group 2, +group 3. *1*) group 1 (intact); *2*) group 2 (negative control); *3*) group 3 (citrocard); *4*) group 4 (fenibut).

RESULTS

In negative control group, volume load increased the rates of contraction (dp/dt+) and relaxation (dp/dt-) by 4 and 5.2%, respectively, and LVP by 5.5%, while in intact animals the corresponding parameters increased by 13.9, 20.9, and 15.6% compared to the initial values (5th sec of observation), which significantly surpassed the corresponding parameters in stressed animals (p<0.05, Table 1).

During maximum isometric load, the studied parameters in stressed animals underwent the following changes: on second 5 the rate of contraction increased by 67.2%, the rate of relaxation by 44.6%, and LVP by 157.9%. In intact animals, the parameters increased by 111.6, 84.4, and 191.1%, respectively, and significantly surpassed the corresponding parameters in negative control (p<0.05, Table 2). The intensity of structure functioning did not differ in the control groups, while MISF in intact animals was higher than in stressed animals by 44.3% (Fig. 1).

In group 3 animals on second 5 of volume load, the rates of contraction and relaxation and LVP increased by 12.2, 10.9, and 12.2%, respectively, compared to initial values, which significantly surpassed the corresponding increase in group 2 (negative control, p<0.05, Table 1). In intact animals, the parameters increased by 83.2, 51.7, and 186.4%, respectively, and significantly surpassed the corresponding parameters in negative control (p<0.05, Table 2). In group 3, MISF increased by 42.9% (p<0.05).

Fenibut administered to stressed animals promoted the increase in the rates of contraction (by 10.1%, p<0.05) and relaxation (by 12.7%, p<0.05) and LVP (by 10.1%) compared to initial values under conditions of volume load (Table 1).

Under conditions of maximum isometric load, the rates of contraction and relaxation in group 4 animals on second 5 after aorta clamping increased by 69.4 and 50% (p<0.05) from the initial values, which differed significantly from the corresponding values in group 2. LVP in group 4 tended to increase by 167.9% (Table 2) and MISF by 12% (Fig. 1). It should be noted that the rates of contraction and relaxation and MISF were significantly lower than in animals receiving citrocard (group 3).

Thus, chronic stress produces a damaging effect on the heart and reduced its functional reserves: this manifested in decreased rates of myocardial contraction and relaxation and LVP in group 2 animals compared to intact rats, which agrees with published data. Citrocard and fenibut administered 60 min before stress considerably reduced the damaging effects of chronic stress: LVP, rates of myocardial contraction and relaxation, and MISF during functional tests increased to a greater extent in animals receiving the test preparation compared to group 2 rats (negative control).

The cardioprotective effect of citrocard and fenibut can be explained by their antistress action at the central and peripheral levels of regulation. The central level of regulation includes limitation of the release of stress hormones, *e.g.* catecholamines and corticotrophin [1,3,8,9], while the peripheral level is determined by some interrelated effects: antioxidant, antiaggregant, and membrane-protective effects, improvement of microcirculation, and stimulation of respiration and oxidative phosphorylation. Our assumptions are based on the results of our previous experiments on dogs demonstrating a de-

TABLE 1. Changes in Myocardial Contractility in Stressed Animals during Volume Load

Group	Initial	Time after load, sec					
		5	10	15	20	25	30
Rate of myocardial contraction, mm Hg×sec^{-1}							
group 1 (intact)	4392.6±1220.1	5076.7±1399.6* (13.9%)	4961.0±1454.9 (12.3%)	4917.6±1362.0 (12.5%)	4845.1±1239.5 (12.3%)	4782.9±1227.6 (11.9%)	4570.9±1376.4 (7.9%)
group 2 (negative control)	4171.5±1389.0	4311.1±1326.8 (4.0%)	4499.2±1255.8 (9.7%)	4458.8±1328.3 (8.0%)	4356.9±1229.4 (6.2%)	4351.3±1124.1 (6.8%)	4250.1±1153.0 (3.9%)
group 3 (citrocard)	3520.8±389.7	3955.2±490.4* (12.2%)	4176.1±443.5 (19%)	4062.8±463.3	3969.0±162.2 (15.5%)	3977.6±266.7 (13.7%)	3629.2±329.9 (13.6%) (3.4%)
group 4 (fenibut)	4277.8±897.0	4797.1±1126.6* (10.1%)	5196.9±1368.4 (17.9%)	5138.4±1536.1 (17.9%)	5143.1±1464.8 (19.3%)	5243.1±1601.4 (22.2%)	5163.9±1612.4 (21.9%)
Rate of myocardial relaxation, mm Hg×sec^{-1}							
group 1 (intact)	2432.2±341.4	3017.4±423.9* (20.9%)	2833.0±459.5 (15.2%)	2767.9±481.3 (13.7%)	2731.9±503.3 (13.3%)	2688.9±405.7 (12.9%)	2638.3±496.7 (11.7%)
group 2 (negative control)	2487.2±978.1	2586.6±883.0 (5.2%)	2709.2±811.0 (11.3%)	2613.1±795.2 (7.4%)	2431.9±722.9 (1.0%)	2568.2±758.9 (6.0%)	2387.7±844.0 (-2.2%)
group 3 (citrocard)	2510.5±315.5	2780.9±329.1* (10.9%)	2831.8±454.7 (12.4%)	2938.7±488.5 (16.8%)	2904.5±388.1 (15.8%)	2898.3±413.1 (15.4%)	2841.6±461.7 (12.9%)
group 4 (fenibut)	2974.5±300.8	3356.5±407.9* (12.7%)	3349.9±658.6 (12.0%)	3377.1±655.8 (13.1%)	3329.3±812.0 (10.9%)	3319.8±767.7 (10.7%)	3247.2±927.4 (7.9%)
LVP, mm Hg							
group 1 (intact)	91.7±21.7	105.7±23.3* (15.6%)	102.8±25.3 (12.0%)	101.5±24.4 (10.6%)	100.8±25.6 (9.6%)	99.2±25.4 (7.8%)	95.6±26.1 (3.6%)
group 2 (negative control)	88.3±21.6	92.8±21.0 (5.5%)	95.0±20.2 (8.6%)	93.7±19.5 (7.4%)	90.9±18.0 (4.5%)	88.7±18.6 (1.8%)	87.3±17.8 (0.3%)
group 3 (citrocard)	74.7±22.2	83.7±24.5* (12.2%)	86.2±24.3 (15.7%)	85.6±24.3 (14.9%)	81.6±25.9 (8.6%)	81.4±22.6 (9.4%)	79.2±22.1 (6.4%)
group 4 (fenibut)	114.4±21.6	125.6±21.6 (10.0%)	128.3±20.9 (12.5%)	134.0±24.1 (17.3%)	131.0±26.2 (14.4%)	129.6±27.6 (13.1%)	130.7±29.0 (14.1%)

Note. Increase from the control level is shown in parentheses. *$p<0.05$ compared to negative control (Newman—Keuls q test)

TABLE 2. Changes in Myocardial Contractility in Stressed Animals under Conditions of Maximum Isometric Load

Group	Initial	Time after load, sec					
		5	10	15	20	25	30
Rate of myocardial contraction, mm Hg×sec^{-1}							
group 1 (intact)	3115.2±1081.1	6515.1±1962.8* (111.6%)	6329.6±1507.0 (109.3%)	5356.6±1322.1 (79.4%)	5239.7±1021.6 (75.8%)	4792.4±710.6 (63.0%)	4605.9±823.7 (54.6%)
group 2 (negative control)	3470.2±887.5	5775.2±1335.0 (67.2%)	5277.7±1189.8 (53.9%)	4938.1±1064.7 (45.6%)	4810.5±1032.3 (41.9%)	4588.7±1229.0 (34.0%)	4762.5±1168.4 (39.9%)
group 3 (citrocard)	3184.8±681.3	5826.4±1192.7* (83.2%)	5572.1±1270.2 (74.6%)	5303.1±1195.3 (66.3%)	5201.9±1230.7 (63.0%)	4554.5±996.3 (43.3%)	4587.1±1250.2 (43.0%)
group 4 (fenibut)	3688.3±834.3	6261.5±1490.9*+ (69.4%)	6272.1±1496.8 (69.7%)	5502.8±1143.5 (49.7%)	5391.4±949.5 (47.5%)	5032.8±836.6 (37.9%)	5095.1±688.0 (40.4%)
Rate of myocardial relaxation, mm Hg×sec^{-1}							
group 1 (intact)	1851.7±397.5	3388.9±592.7* (84.4%)	3223.1±661.0 (74.8%)	2791.0±413.5 (52.8%)	2664.1±280.1 (47.1%)	2458.0±282.1 (35.3%)	2377.9±332.5 (30.3%)
group 2 (negative control)	1908.5±375.0	2746.8±471.8 (44.6%)	2652.5±438.7 (40.1%)	2465.8±432.8 (29.8%)	2341.0±382.7 (23.7%)	2282.0±351.4 (20.9%)	2220.0±376.3 (17.0%)
group 3 (citrocard)	2186.5±545.6	3433.2±876.9* (57.1%)	3240.8±868.6 (47.9%)	3120.9±1000.0 (42.5%)	3006.0±926.5 (37.6%)	2811.7±872.9 (28.4%)	2755.0±874.6 (25.2%)
group 4 (fenibut)	2206.6±378.4	3300.8±492.7*+ (50.0%)	2873.1±304.3 (31.4%)	2658.1±299.1 (21.4%)	2593.4±328.7 (18.2%)	2544.6±245.6 (16.6%)	2496.1±250.1 (14.5%)
LVP, mm Hg							
group 1 (intact)	68.3±13.0	197.7±29.8* (191.1%)	194.1±24.5 (187.1%)	172.6±18.0 (156.9%)	170.3±19.1 (153.2%)	164.2±19.9 (144.5%)	161.4±20.3 (140.3%)
group 2 (negative control)	70.9±14.4	182.1±32.7 (157.9%)	179.2±27.0 (155.9%)	174.5±25.6 (149.9%)	171.1±27.1 (144.6%)	165.1±33.1 (134.6%)	162.9±35.0 (131.4%)
group 3 (citrocard)	68.9±5.6	197.1±16.0* (186.4%)	187.9±19.2 (172.8%)	181.0±19.3 (163.3%)	182.8±16.0 (165.8%)	170.7±22.0 (147.6%)	165.1±24.3 (138.9%)
group 4 (fenibut)	76.5±11.3	205.2±34.6 (167.9%)	193.5±30.9 (153.2%)	187.2±14.0 (146.4%)	185.1±14.7 (143.6%)	185.2±13.7 (143.8%)	186.0±14.1 (144.9%)

Note. Increase from the control level is shown in parentheses. *p<0.05 compared to: *negative control, +group 3.

crease in concentration of LPO products (diene conjugates, crotonic dialdehyde and MDA) in the ischemic heart under the effect of citrocard [6]. The test preparation inhibiting LPO prevent destruction of membranes of cardiomyocytes and their organelles (*e.g.* mitochondria), thus preserving the function of the energy production system and contractile apparatus of these cells. Citrocard and fenibut enhance the resistance of cell membranes to other factors, *i.e.* exhibit membrane-protective properties, and inhibit ADP-induced platelet aggregation, which probably improves microcirculation disturbed by stress exposure. Previous experiments also showed that citrocard and fenibut prevented microcirculatory disturbances induced by chronic alcoholization and inhibited reduction of linear and volume blood flow rates in mesenterial vessels [4].

Thus, chronic (2 weeks) stress reduced functional reserves of the heart, which manifested in disturbed functioning of the contractile apparatus of cardiomyocytes (decrease in the rates of contraction and relaxation and LVP in comparison with those in intact animals) under conditions of functional tests. GABA derivatives citrocard (50 mg/kg) and, to a lesser extent, fenibut (50 mg/kg) prevented pathological changes in the myocardium and protected its functions, *i.e.* exhibited a cardioprotective effect.

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