of respiration. Such a conclusion holds true only for pronounced paradoxical phenomena.

REFERENCES

1. T. N. Bodrova, A. I. Korzilov, and F. F. Tetenev, *Byull. Eksp. Biol. Med.*, **115**, No. 1, 20-21 (1993).
2. F. F. Tetenev, *Ibid.*, **81**, No. 1, 30-32 (1976).
3. F. F. Tetenev, *Ibid.*, **85**, No. 1, 264-267 (1978).
4. F. F. Tetenev, *Biomechanics of Respiration* [in Russian], Tomsk (1981).
5. P. Machlem and B. Murphy, *Am. J. Med.*, **57**, No. 10, 371-377 (1974).
6. W. Stead, D. Fry, and R. Ebert, *J. Lab. Clin. Med.*, **40**, 674-681 (1952).
7. W. Webb, J. Smith, and G. Campbell, *Ann. Surg.*, **153**, No. 5, 650-657 (1961).

Effect of Preliminary Adaptation to Transauricular Electrostimulation on the Content of Catecholamines and Met-Enkephalin in Rat Heart and Adrenals in Stress and Acute Myocardial Infarction

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Adaptation to transauricular electrostimulation decreases the content of epinephrine in the adrenal glands and norepinephrine in the heart. Immobilization stress has no appreciable effect on the content of catecholamines in the heart and adrenal glands. In animals with myocardial infarction, the content of norepinephrine in the heart decreases 2-fold, while the content of epinephrine in the adrenals decreases inconsiderably. Adaptation to transauricular electrostimulation is associated with a rise in met-enkephalin concentration. Preadaptation induces a more pronounced rise of met-enkephalin and promotes normalization of epinephrine in the adrenals, without changing the content of norepinephrine in the heart.

Key Words: *transauricular electrostimulation; stress; myocardial infarction; catecholamines; met-enkephalin*

Recent experimental data suggest that elimination of the stress component of the damage partially underlies the protective effect of transauricular electrostimulation (TES) in acute myocardial infarction [2]. This assumption is confirmed by the fact that TES can limit the stress reaction through suppression of adrenoreactivity and activation of the opioid systems. Indeed, similarly to adaptation to stress, preadaptation to TES reduces heart sensitivity to toxic concentrations of epinephrine [4] and considerably elevates the plasma content of immunoreactive β-endorphin in rats [3]. However, the role of the interplay between catecholaminergic and opioid systems in protective effect of TES is poorly understood. The effect of TES on the interaction between these systems in the adrenal glands is also little studied.

In the present study we evaluate the effect of TES on the content of catecholamines and met-enkephalin (ME) in rat heart and adrenal glands in immobilization stress and acute myocardial infarction.

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MATERIALS AND METHODS

Experiments were performed on 45 male Wistar rats weighing 250 g. The animals were divided into 8 groups: intact rats (control); adaptation to TES; immobilization; TES+immobilization; false myocardial infarction (sham-operation without ligation of the coronary artery); TES+false myocardial infarction; 2-day-old myocardial infarction; TES+myocardial infarction.

Immobilization stress was modeled by fixing the animals for 1 h in the supine position with fasten extremities. Experimental myocardial infarction was modeled according to the method of Selye [11] and verified by ECG. Adaptation to TES consisted of 10 daily sessions and was performed as described elsewhere [1]. The animals were decapitated under Nembutal narcosis immediately after the course of TES or exposure to stress or 2 days after modeling myocardial infarction or sham-operation. The heart (without atria) and adrenal glands were isolated, frozen in liquid nitrogen, and stored at -30°C for no more than 1 week. The specimens were weighted on electron balances before extraction. The content of norepinephrine (NE) in the heart and epinephrine and ME in the adrenal glands was determined. For enkephalin assay the tissue was extracted with 0.1 M HCl, while catecholamines were extracted with 0.1 M $HClO_4$ (with 0.004% sodium hyposulfite) at a 1:10 w/v ratio. The tissues were homogenized with a cavitation-ultrasonic tissue disintegrator (Ultraturrax), the homogenates were centrifuged at 15,000*g* and 4°C for 20-30 min. The resultant supernatant was filtered through 0.2-μ filters and used in the analysis. Enkephalins were analyzed by radioimmunoassay using commercial kits (Technological Alliance, Russia), catecholamines were measured by reverse-phase high-performance liquid chromatography.

Chromatographic system consisted of a standard analytic column with a Separon 18.5 carrier, a LC-4B electrochemical detector (BAS), plunger pump, and a Chromatopac CR-6A integrator (Shimadzu). Working electrode was settled at 700 mV against reference electrode. Elution rate was 0.8 ml/min. Mobile phase (pH 3.0-3.1) contained 14.1 g monochloroacetic acid, 252 mg sodium EDTA, 200 mg sodium octanesulfonate as a ion-pair reagent, and 20 ml acetonitrile per 1 liter bidistilled water. Epinephrine and NE standard were from Sigma.

The data were processed by ANOVA (Student's *t* and Mann—Whitney tests) using Stat & Graph Corp. software.

RESULTS

Repeated TES reduced the content of NE in the heart and epinephrine in the adrenals by 38 and 39% of the control values, respectively, while the content of ME increased by approximately 48% (Table 1). These data suggest that TES had a pronounced effect on peripheral sympathicoadrenal and opioidergic systems in intact rats. However, the increased content of ME in the adrenals, which is stored in the chromaffin granules and secreted together with epinephrine [10], suggests that the dramatic decrease of epinephrine in the adrenals cannot be explained by its enhanced excretion (and consequently, the rise of its blood concentration). This assumption is confirmed by the fact that repeated TES did not reduce the weight of the adrenals, i.e., long-term enhanced release of epinephrine did not exhaust the adrenal system. Inhibition of the synthesis and excretion of epinephrine seems to be more likely. Similar mechanisms may underlie the marked decrease of NE in the heart after repeated TES. These findings agree with the data on reduced NE content in brain struc-

TABLE 1. Content of NE and Epinephrine (μg/g Tissue) and ME (pmol/g Tissue) in Rat Heart and Adrenal Glands (*M*±*m*)

Group	Heart	Adrenal glands	
	norepinephrine	epinephrine	met-enkephalin
Control	1.68±0.15	402±82	19±1
TES	1.04±0.11*	245±11*	28.2±3.7*
Stress	1.73±0.16	392±33	25.3±2.1*
TES+stress	1.46±0.12^{x}	331±27^{x}	40.9±7.2*
Sham-operation	0.97±0.16*	291±16	44.3±11.0*
TES+sham-operation	1.01±0.24*	383±21**	89.0±22.6^{+}
Infarction	0.47±0.10*,**	298±51	26.4±5.6
TES+infarction	0.51±0.14*	353±20**	49.8±6.1°

Note. $p<0.05$: *in the control, **in sham-operation, ^{x}in TES, $^{\circ}$in infarction (Student test); $^{+}$in sham-operation (Mann—Whitney test).

tures after repeated electroacupuncture [8] not accompanied by changes in the blood NE level and suggest that preliminary adaptation to TES reduces the tone of the sympathicoadrenal system.

The increased content of ME in the adrenal glands after TES also indicates activation of the local ME pool and probably plays a role in the regulation of catecholamine metabolism and release from the adrenals. However, experimental data are not sufficient to understand the mechanism of this process. It cannot be excluded that the epinephrine content decrease was influenced by activation of the β-endorphin system. Previous studies [3] have demonstrated a more than 2-fold elevation of plasma β-endorphin in rats subjected to repeated TES. This assumption is based on the well established fact that morphine reduces the content of epinephrine the adrenal gland medulla [7]. It can be hypothesized that the decrease of NE in the heart after adaptation to TES is a consequence of activation of the opioid systems (including pituitary and adrenal glands), which is considered to be an important factor restricting activation of the adrenergic system during stress [6].

As seen from Table 1, analogously to TES, a 1-h immobilization stress significantly increased the adrenal content of ME by 33% in comparison with the control. However, stress had no effect on the level of NE and epinephrine and did not reduce the weight of adrenals. Similar results were obtained when the content of epinephrine was measured 2 h after immobilization stress [9]. This may be due to the fact that short-term immobilization (as well as electroshock [12]) almost equally stimulates synthesis and utilization of catecholamines. Unlike short-term stress, a more intense and prolonged stress (pain-emotional) probably disturbs this balance, which results in a decrease in the heart NE content [5]. Operation trauma (sham-operation), which induces a considerable (by 43%) decrease in cardiac NE in comparison with the control (Table 1), also can be regarded as a severe stress.

Although stress is an inevitable component of myocardial infarction, neither infarct nor sham-operation reduced the content of epinephrine in the adrenals. No changes in the weight of adrenals characteristic of severe stress damages (H. Selye, 1953) were seen in both animal groups. On the other hand, infarction induced a marked decrease in cardiac NE, which surpassed 2-fold the decrease observed after sham-operation (Table 1). It should be noted that the level of ME was increased after both myocardial infarction and sham-operation ($p<0.05$), which can be considered as an indicator of activation of the ME system.

Preadaptation to TES did not prevent the drop of heart NE in sham-operated animals and in myocardial infarction, but it significantly increased the epinephrine content in the adrenals (by 38 and 18%, respectively) almost to the normal level and induced an approximately 2-fold rise of ME in the adrenals. TES carried out before immobilization stress had practically no effect on the level of catecholamines in the heart and adrenal glands, but sharply increased the content of ME, this increase being 1.5-fold greater than in "stress" and "TES" groups.

These data suggest that first, preadaptation to TES has presumably no effect on the intensity of epinephrine accumulation in the adrenals and NE in noradrenergic terminals in the heart in acute stress and infarction, but inhibits epinephrine release from the adrenals. Second, the rise of ME concentration in the adrenals in all studied interventions indicates a high lability and great reserve capacity of the adrenal ME system, which suggest that this system is an important regulator of metabolism and secretion of catecholamines. Indeed, in our experiments adaptation to TES, stress and ischemic damage markedly increased the level of ME, while the effect of the combination of TES with stress and infarction was the sum of individual influences. The rise of ME in the adrenals was accompanied by recovery or maintenance of the normal (control) epinephrine level.

Thus, our data suggest that the protective effect of TES in myocardial infarction and stress is associated predominantly with restriction of the hormonal component of the sympathicoadrenal system; the adrenal ME system activated by preadaptation to TES being involved in this regulation. This apparently prevents the stress-induced damage. However, this assumption requires a more detailed study of the effect of TES on catecholamine and ME metabolism.

REFERENCES

1. L. M. Belkina, O. P. Budanova, N. B. Korchazhkina, *et al.*, *Byull. Eksp. Biol. Med.*, **120**, No. 12, 568-571 (1995).
2. F. Z. Meerson, *Adaptation Medicine. Mechanisms and Protective Effects of Adaptation* [in Russian], Moscow (1993).
3. F. Z. Meerson, M. G. Pshennikova, B. A. Kuznetsova, *et al.*, *Byull. Eksp. Biol. Med.*, **117**, No. 1, 16-18 (1994).
4. F. Z. Meerson, S. A. Radzievskii, V. I. Vovk, *et al.*, *Kardiologiya*, No. 10, 72-77 (1991).
5. F. Z. Meerson, S. A. Radzievskii, L. M. Giber, *et al.*, *Dokl. Akad. Nauk SSSR*, **237**, No. 4, 977-980 (1977).
6. M. G. Pshennikova, *Pat. Fiziol.*, No. 3, 86-90 1987).
7. H. Henderson, J. Hages, and H. V. Kosterlitts, in: *Release of Catecholamines from Adrenergic Neurons*, D. M. Paton (Ed.) [Russian translation], Moscow (1982), pp. 207-215.
8. X. J. Cao, B. B. Xu, and W. X. Lu, *Acupunct. Electrother. Res.*, **8**, 25-55 (1983).
9. G. M. Gilad and D. C. Jimerson, *Brain Res.*, **206**, 83-93 (1981).
10. J. V. Lewis, M. J. Tordoff, J. E. Sherman, and J. C. Liebeskind, *Science*, **217**, 557-559 (1982).
11. H. Selye, E. Bajuss, S. Grasso, *et al.*, *Angiology*, **11**, 398-407 (1980).
12. E. A. Stone, L.-S. Freedman, and L. E. Morgano, *Pharmacol. Biochem. Behav.*, **9**, 551-553 (1978).