Role of Cholinergic Regulation of the Heart in the Protective Antiarrhythmic Effect of Adaptation to Continuous Moderate Stress

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Rats were adapted to the continuous action of moderate immobilization stress for 1, 5, and 15 days. Thereafter the threshold of ventricular fibrillation and the heart rate were compared with biochemical indexes of adrenergic and cholinergic regulation of the heart, namely, catecholamine, cAMP, and cGMP content, acetylcholinesterase and choline acetyltransferase activity, the number and affinity of cardiac muscarinic receptors, and the catecholamine content in the adrenals. The threshold of ventricular fibrillation fell on the 1st day due to a predominance of the adrenergic regulatory effect over the cholinergic. Adaptation for 5 days is attended by a rise of the threshold of ventricular fibrillation to the norm and by marked bradycardia, both these shifts being abolished by atropine. Elevation of the heart's resistance to arrhythmias stems from the prevalence of cholinergic regulation. Equilibrium between the cholinergic and adrenergic effects on the heart was found as a result of 15-day adaptation. The normal threshold of ventricular fibrillation and the increased cardiac resistance to arrhythmia were preserved and dictated largely by adaptive changes at the cardiomyocyte level.

Key Words: *stress; adaptation; cholinergic regulation; heart*

The development of atropine-dependent bradycardia during adaptation to continuous stress was established previously. Such adaptation is attended by an increase of cardiac resistance to ischemic and reperfusion arrhythmias. It is thought that this antiarrhythmic effect of adaptation is associated with an enhancement of the cholinergic tonic influence on the heart [8,9]. For elucidation of this mechanism a comparison study was performed in rats between the dynamics of physiological indexes of the tonic chronotropic effects of the parasympathetic system on the heart and the electrical stability of the heart, characterizing its vulnerability to arrhythmogenic impacts, and the dynamics of the biochemical indexes attesting to the state of the cholinergic and adrenergic regulation of the heart.

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MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 200-250 g. Continuous stress was produced by restricting the animals' movements due to placement in individual cages the size of a pencil-case (a relatively mild immobilization stress). Food and water were provided *ad libitum.* The animals were taken for the experiment 1, 5, and 15 days after caging. In the physiological part of the study the intensity of the tonic chronotropic

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TABLE 1. Dynamics of Catecholamine Concentration (in μg/g) in Rat Myocardium and Adrenals in Adaptation to Continuous Stress

Index	Control (12)	Adaptation		
		1 day (12)	5 days (8)	15 days (10)
Myocardium				
Norepinephrine	0.92±0.05	0.35±0.06***	0.59±0.14*	0.76±0.12
Epinephrine	0.13±0.02	0.10±0.01	0.29±0.13	0.23±0.02**
Dihydroxyphenylalanine	0.33±0.09	0.64±0.10*	0.87±0.23*	0.30±0.16
Dopamine	0.18±0.09	0.25±0.11	0.22±0.11	0.27±0.13
Total content of catecholamines (norepinephrine + epinephrine)	1.04±0.04	0.45±0.06***	0.87±0.05*	0.98±0.13
Adrenals				
Norepinephrine	229±78	151±42	475±72*	0
Epinephrine	797±64	288±32**	580±74*	330±39**
Dihydroxyphenylalanine	178±11	617±68**	160±15	178±26
Dopamine	2.7±1.0	6.3±3.0	3.7±1.9	16.7±2.4**
Norepinephrine + epinephrine	1026±72	439±39**	1055±73	330±39***

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ as compared to the control. The number of animals is given in parentheses.

effect of the parasympathetic system on the heart was estimated in an acute experiment *in situ* according to the difference between the heart rate after and before the administration of atropine sulfate (1 mg/kg intraperitoneally). The electrical stability of the heart was assessed by the value of the threshold of ventricular fibrillation. For this purpose the apex of the heart was stimulated in rats under urethan anesthesia (1.6 g/kg, intraperitoneally) for artificial pulmonary ventilation, as described elsewhere [6]. The threshold of ventricular fibrillation was determined as the minimal value of current intensity at which fibrillation appeared. In another part of the study rats were decapitated at the mentioned times, the heart and adrenals were removed, and the separated atria were placed in liquid nitrogen. The activity of choline acetyltransferase (CAT, EC 2.3.1.6) was determined in these samples by the radiosubstrate method [7], the activity of acetylcholinesterase (ACE, EC 3.1.1.7) and butyryl cholinesterase (BCE, EC 3.1.1.8) by photometry [1], the cAMP and cGMP content was measured by radioimmune assay with Amersham kits, and the number and affinity of muscarinic choline receptors by the radioligand method [15] using ^{3}H-quinuclidinyl benzylate (Amersham). Catecholamine content was measured in the left ventricle and in the adrenals by the fluorimetric technique [2].

RESULTS

It was shown that the heart rate did not differ from the control on the 1st day after stress. In this case the threshold of ventricular fibrillation characterizing the heart's vulnerability to arrhythmogenic factors dropped (to 4.1±0.57 mA versus 7.84±0.24 mA in the control), as is typical for acute stress [3]. These shifts were attended, as shown in Tables 1 and 2, by a nearly 3-fold drop of the norepinephrine content in the myocardium and of epinephrine in the adrenals, by an elevation of the dihydroxyphenylalanine content in both organs, by a 23% cAMP increase in the myocardium, and by a nearly twofold decrease of cGMP, attesting to marked activation of the adrenergic system and its effect on the heart, in conformity with data reported on an increase of the catecholamine "output" in stress [12]. Table 2 shows that ACE and BCE activity increased by 31 and 18%, respectively, in the myocardium during this period, while the density of muscarinic choline receptors diminished by 27%. These facts point to the known increase of acetylcholine "output" in the myocardium and are in agreement with the data on the activation not only of adrenergic but also of cholinergic regulation in acute stress [13,14]. However, the decrease of the CAT activity, the rise of the cAMP level, and the reduction of cGMP in the myocardium along with the data on the catecholamine content suggest that adrenergic effects on the heart dominate over cholinergic effects during the 1st day of stress.

A marked bradycardia evolving on the 5th day of stress was abolished by atropine and, consequently, resulted from an enhanced chronotropic cholinergic effect on the heart. Quantitatively this

effect was 2.2-fold increased, since the heart rate rose after atropine to 136 beats/min in adapted rats versus 62 beats/min in the control. In this case the threshold of ventricular fibrillation rose to the control level and this increase was eliminated by atropine. These shifts took place, as is evident from Tables 1 and 2, against the background of a norepinephrine rise in the heart to 64% of the control, a 2.5-fold increase in the dihydroxyphenylalanine content, and a cAMP drop to the control level. The level of epinephrine in the adrenals was raised to 73% of the baseline value and the norepinephrine content was doubled while dihydroxyphenylalanine and dopamine were no different from the control. The data on catecholamine changes denote a weakened activation of the adrenergic system in stress. As is obvious from Table 2, the activity of ACE and BCE did not differ from the control, while CAT activity remained lowered, attesting to normalization of the transmitter level in the heart. At the same time, the number and affinity of muscarinic choline receptors rose by 13 and 57%, respectively, as compared to the control. These data, together with the decrease of the catecholamine output in the heart and from the adrenals and the marked atropine-dependent bradycardia, imply a decrease of the adrenergic and a predominance of the cholinergic effects on the heart.

After 15 days of adaptation to stress the heart rate and the threshold of ventricular fibrillation did not differ from the control. This was combined (Tables 1 and 2) with normalization of the content of norepinephrine, dihydroxyphenylalanine, and dopamine in the myocardium along with conservation of the normal level of cAMP and a cGMP reduction, testifying to normalization of the sympathetic regulation of the heart. An increase of the dopamine level in the adrenals together with lowered norepinephrine and epinephrine and a normal level of dihydroxyphenylalanine may signal disruption of catecholamine synthesis at the stage of dopamine-norepinephrine conversion. This calls for further analysis. The activity of ACE did not differ from the control, while CAT remained below it during this period. The density and affinity of muscarinic choline receptors were elevated by 20 and 65% above the control values, respectively. Coupled with the normal values of the heart rate, catecholamines, and cAMP content in the myocardium, these data may signify that by the 15th day of adaptation a certain equilibrium is established between the adrenergic and cholinergic effects on the heart.

Thus, we can assume that the fall of the threshold of ventricular fibrillation and the previously reported [4] increase of the heart's vulnerability to ischemic and reperfusion arrhythmias observed during the 1st day of stress are largely determined by the predominance of adrenergic over cholinergic effects on the heart. The restoration of the fibrillation threshold and increase of the cardiac resistance to arrhythmias [4], appearing as a result of 5-day adaptation, were obviously due to a prevalence of cholinergic effects in heart regulation. What is the mechanism of this "cholinergic antiarrhythmic protection"? Our data suggest that it has to do with an enhanced extracardial cholinergic effect on the heart and a growth of the number and affinity of muscarinic choline receptors sensitive to this influence. The increase of the cholinergic effects may stem from activation of the stress-limiting systems, notably the GABA-ergic and opioidergic. The GABA system [6,11] and the opioid system [6] have been shown to be activated in adaptation to stress and may not just limit the activity of the adrenergic centers of heart regulation [6], but even stimulate the cholinergic cen-

TABLE 2. Dynamics of Activity of Enzymes of Acetylcholine Metabolism (CAT, ACE, and BCE), Density and Affinity of Muscarinic Choline Receptors (MCR) and cAMP and cGMP Content in Rat Atria during Adaptation to Continuous Stress

Index	Control (10)	Duration of adaptation		
		1 day (10)	5 days (10)	15 days (8)
CAT activity, pmol/secxg tissue	370±5	320±3*	301±2*	312±5*
ACE activity, pmol/secxg tissue	632±32	829±62*	732±46	558±46
BCE activity, pmol/secxg tissue	476±27	563±25*	464±33	384±25*
Density of MCR, fmol/mg protein	123±2	90±2*	138±3*	147±2*
K_d, nM	1.24±0.04	0.78±0.04*	0.54±0.03*	0.43±0.03*
cAMP concentration, pmol/g tissue	703±25	866±40*	754±7	644±77
cGMP concentration, pmol/g tissue	37.0±5.8	18.3±2.4*	22.5±5.0	13.7±2.1*

Note. * = differences from control are reliable; the number of animals is given in parentheses.

ters [9]. As for the muscarinic choline receptors, they are known to be simultaneously coupled in cardiomyocytes with both phospholipase C and the adenylate cyclase coupled with the β-adrenoceptors and with the K^+ channels which are responsible for K^+ discharge from the cell. Therefore, stimulation of the muscarinic choline receptors by acetylcholine boosts the K^+ outflow from the cardiomyocyte and also inhibits adenylate cyclase. This limits the cAMP-dependent Ca^{2+} entry into the cardiomyocyte induced by catecholamines via the β-receptors [10]. Both these shifts lead to the development of hyperpolarization and to a decrease of the cardiomyocyte membrane excitability [8,10], this being an antiarrhythmic factor.

As has been shown by our data, the increase in the resistance of the heart to arrhythmogenic factors in 15-day adaptation [4] was not accompanied by an enhancement of the cholinergic effects on the heart, but was realized against the background of a certain balance between the adrenergic and cholinergic effects. The protective effect of such adaptation may derive from two circumstances. First, during this period the increased density and affinity of muscarinic receptors and, consequently, their role in the antiarrhythmic effect mentioned above are conserved. Second, it is possible that structural changes develop in cardiomyocytes which have been found in adaptation to repeated stress and are designated as the "phenomenon of adaptive stabilization of structures," which is responsible for the stepped-up cardiac resistance to different alterations including arrhythmogenic factors [5].

On the whole, it may be assumed that the activation of cholinergic regulation plays a key role in the mechanism of the antiarrhythmic effect of adaptation to continuous moderate stress and is realized both on the central level and locally in the heart.

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