INCREASED α_1-ADRENORECEPTOR ACTIVITY OF THE RAT HEART DURING ADAPTATION TO INTERMITTENT HYPOXIA

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Adaptation to intermittent hypobaric hypoxia limits damage to the heart and the onset of arrhythmias in acute experimental myocardial infarction and postinfarction cardiosclerosis [3] and also disturbances of energy metabolism and cardiac contractility in acute anoxia and subsequent reoxygenation [1]. However, the problem of the mechanism of this phenomenon has not yet been resolved.

Accordingly, the aim of the investigation described below was to study the state of the β-receptor — adenylate cyclase system and also of the Ca^{2+}-mobilizing α-adrenoreceptors and certain other receptors of the heart during adaptation of rats to intermittent hypoxic hypoxia.

EXPERIMENTAL METHOD

Adaptation to intermittent hypoxia was carried out under pressure chamber conditions for 40 days, for 4 h daily, to an "altitude" of 4000 m. The course of adaptation began at 1000 m, and the "altitude" was increased in steps to 4000 m by the 8th day. Tests were carried out 20 and 40 days after the beginning of the course of adaptation.

Plasma membranes were isolated from the heart by the method in [4] with certain modifications. Plasma membranes for the study of ligand binding with dihydropyridine receptors of voltage-dependent Ca^{2+}-channels were isolated from the rats' hearts by the method in [6]. Binding of [^{3}H]-dihydroalprenolol with β-adrenergic receptors was measured by the method in [9] with certain modifications, and binding of [^{3}H]-prazocin with α_1-adrenoreceptors by the method in [10], whereas binding of [^{3}H]-quinuclidinyl benzylate with muscarinic acetylcholine receptors was measured by the method in [12]. Binding of [^{3}H]-PN-200-110 with dihydropyridine receptors of the voltage-dependent Ca^{2+}-channel was carried out by the method in [6] with certain modifications. The reaction of binding of ligands with receptors was stopped by the rapid addition of 15 ml of cold buffer of the same composition and pH, at 4°C, followed by filtration through CF/C filters ("Whatman," England). The filters were placed in flasks with dioxan scintillator and their radioactivity was determined on a "RackBeta" scintillation counter (LKB, Sweden). The number of receptors (B_{max}) and dissociation constants of the ligand (K_d), the reciprocal of receptor affinity, was calculated on a personal computer using the EBDA/Ligand program for an IBM-PC (McPherson, 1984). Adenylate cyclase (AC) activity was determined by the method described by Tkachuk and Baldenkov [4]. To determine changes in AC activity in response to stimulation, L-isoproterenol (0.1 mM) or sodium fluoride (0.1 mM) was added to the incubation mixture. The [^{32}P]-cAMP formed was separated on columns with aluminum oxide. Radioactivity was counted by using the Cherenkov effect. All experiments were conducted in three parallel tests. Protein was determined by the method in [11].

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TABLE 1. Number of β-Adrenoreceptors and AC Activity in Rat Heart Membranes during Adaptation to Hypobaric Hypoxia

Experimental conditions	β -adrenergic receptors		AC activity, pmoles/mg protein/min		
	B_{max}, fmoles/mg protein	K_d, nM	basal activity	Response to L-isoproterenol	response to NAF
Control	51,3±2,0	2,20±0,1	1,54±0,03	3,53±0,05	11,12±0,34
Adaptation to hypoxia					
20 days	67,9±2,8**	2,08±0,2	1,43±0,05	3,79±0,08*	15,96±0,13**
40 days	64,3±4,0**	2,36±0,3	1,00±0,10**	2,80±0,04**	10,0±0,64

Legend. Significance of differences: *$p < 0.05$, **$p < 0.01$.

TABLE 2. Number of α_1-Adrenoreceptors Muscarinic Acetylcholine Receptors, and Dihydropyridine Receptors of Voltage-Dependent Ca^{2+} Channels in Rat Heart during Adaptation to Hypobaric Hypoxia

Experimental conditions	α_1-adrenergic receptors		Muscarinic acetylcholine receptors		Dihydropyridine receptors of Ca^{2+}-channels	
	B_{max}, fmoles/mg protein	K_d, pM	B_{max}, fmoles/mg protein	K_d, pM	B_{max}, fmoles/mg protein	K_d, pM
Control	132±18	444±22	366±9	150±14	235±11	130±8
Adaptation to hypoxia						
20 days	196±19*	269±34*	506±13*	321±30	299±25*	181±21*
40 days	157±30	255±52*	450±15*	451±53*	259±9	147±14

Legend. Significance of differences: *$p < 0.01$.

EXPERIMENTAL RESULTS

The results in Table 1 show that during adaptation of rats to intermittent hypobaric hypoxia the number of β-receptors in the myocardial plasma membranes rose after 20 days by 32%, and remained rather high until the end of the course of adaptation, while the affinity of these receptors for the ligand used was unchanged throughout the course of adaptation. Basal AC activity on the 20th day of adaptation showed a tendency to fall, and by the end of the course of adaptation it was significantly lower by 35% ($p < 0.01$); the response to stimulation by the β-selective agonist L-isoproterenol also was depressed at the same time. Isoproterenol-stimulated AC activity at this stage of the experiment was 79% of its value in the control group. The increase in AC activity in response to the action of sodium fluoride, reflecting the potential possibilities of the response of AC to stimulating factors, was almost 1.5 times higher after 20 days of adaptation than in the control, and by the end of the course of adaptation the absolute value of fluoride stimulated AC activity did not differ from that in the control animals.

Thus at the end of the course of adaptation of the rats to hypoxia some increase was observed in the number of β-receptors in the heart, accompanied by a simultaneous decrease in basal AC activity, and depression of its response to the stimulating action of the β-agonist. This effect was observed while the maximal response of AC, detected with the aid of sodium fluoride, was preserved. The decrease in activity of the β-receptor — AC system during adaptation to hypoxia which we found is in agreement with earlier observations [2], which showed that adaptation to intermittent hypoxia considerably increases the resistance of the heart to isoproterenol damage.

It will be clear from the data in Table 2 that after 20 min of adaptation to hypoxia an increase of 48% was observed in the number of α_1-adrenoreceptors, and an almost twofold increase in affinity for the ligand. After 40 days the number of receptors decreased a little, in the direction of normal, but affinity for the ligand still remained almost doubled. Thus as a result of adaptation to periodic hypoxia an increase in α_1-adrenoreceptor activity of the heart developed. This last fact may play an important role in adaptation of the heart to intermittent hypoxia, leading to improvement of myocardial contractility. The positive inotropic effect of physiological α_1-agonists is known to be realized, besides through AC, chiefly on account of an increase in outflow of Ca^{2+} from depots in the sarcoplasmic reticulum into the sarcoplasm [13].

Besides its positive inotropic action, stimulation of α_1-adrenoreceptors of the heart participates in the regulation of metabolism, by increasing activity of phosphofructokinase [5], the chief component limiting the velocity of glycolysis, and which in turn may help to maintain the contractile function of the heart under hypoxic conditions. This is in agreement with our previous findings [1], showing that restoration of the contractile function of the heart and of the creatine phosphate and ATP levels in the myocardium during reoxygenation after acute anoxia in rats adapted to intermittent hypoxia is observed much more rapidly than in unadapted rats.

The number of muscarinic acetylcholine receptors was increased by 38% as early as after 20 days (Table 2), and towards the end of the course it remained significantly increased, although their affinity for the ligand at this stage was reduced. This increase in the number of muscarinic acetylcholine receptors may have a protective action of dual nature: first, by changing the state of receptor-dependent K^+-channels, it may affect myocardial excitability [8], and second, it may facilitate realization of inhibitory influences on AC activity which, as we showed, is significantly depressed toward the end of the adaptation period. This may limit the activating effect of catecholamines on voltage-dependent Ca^{2+}-channels and may inhibit the entry of Ca^{2+} into the cardiomyocytes [8].

It is also clear from Table 2 that the number of voltage-dependent Ca^{2+}-channels in the heart, determined as the number of dihydropyridine receptors, was increased by 27% on the 20th day of adaptation, but by the end of the period of adaptation it returned to normal, i.e., a definite similarity was observed between the time course of the change in the number of voltage-dependent Ca^{2+}-channels and of α_1-receptors in the myocardium.

It can thus be tentatively suggested that on the whole the reduction of activity of β-receptor — AC system and the increase in α_1-adrenoreceptor activity of the myocardium which we found may together play a definite role in the cardioprotective mechanism of adaptation to intermittent hypoxia.

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