ROLE OF NORADRENALIN IN REGULATION OF MYOCARDIAL OXIDATIVE METABOLISM IN RATS DIFFERING IN RESISTANCE TO HYPOXIA

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In animals differing in sensitivity to hypoxia this feature applies at both organ and cellular levels [2]. For instance, in the brain of animals with high (HR) and low (LR) resistance to oxygen deficiency, resistant neurons and neurons sensitive to hypoxia respectively have been shown to be present in the cerebellum [3]. These properties are due to fundamental differences in their oxidative metabolism. The same has been shown also for the heart: disturbances of myocardial function of LR rats under conditions of oxygen deficiency develop much sooner and are significantly more severe than in the myocardium of LR rats [1, 2], a feature which is linked to the initially different metabolic properties and, in particular, the nature of their oxidative metabolism, determining the regulation of anaerobic and aerobic processes and the maintenance of energy homeostasis of the cardiomyocytes [1, 2]. All these processes, in turn, are under the hormonal control of adrenoreceptors, which evidently take part in their regulation [5].

The aim of this investigation was to study the possibility of influencing the bioenergetics of the myocardium through its adrenoreceptors in animals differing in their resistance to hypoxia.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 250-300 g, divided beforehand into those with low resistance (LR) and those with high resistance (HR) to hypoxia [6], which were used in the experiments two weeks after testing. The animals were anesthetized with ether, the heart removed, and washed in cold 0.14 M NaCl solution, after which it was perfused by Langendorff's method in our own modification [4]. The Krebs–Henseleit perfusion solution was saturated with carbogen at 37°C. The chemicals were added to the reservoir with perfusion solution in volume of not more than 1% of the volume of the perfusion solution. The following parameters were recorded: The heart rate (HR) as the number of apicobasal contractions of the heart per minute (beats/min), the force of the cardiac contractions (FCC), as the amplitude of the apicobasal contractions of the heart with the aid of a strain-gauge transducer per millimeter (10 mm corresponds to a tension of 1 g), and the tension developed by the heart (HR × FCC). The value of pO_2 in the perfusion fluid flowing toward the heart (arterial) and away from it (venous) was measured polarographically with the aid of a Clark electrode ("Radiometer"). The rate of oxygen consumption by the myocardium (respiration) (V_{resp}, in μmoles O_2/min·g dry weight) was calculated; the coro-

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TABLE 1. Values of Various Functional-Metabolic Parameters of Isolated Myocardium of Rats with High (HR) and Low (LR) Resistance after Exposure to Noradrenalin (NA) for 5 min (M ± m)

Parameters		Absolute values		Per cent of control		LR/HR
		LR	HR	LR	HR	
HR, beats/min	C	321±45	279±42	100	100	115
	NA	404±39	397±37	126	142	102
FCC, mg	C	26±3*	39±4	100	100	66
mg	NA	16±1,2*	30±2,5	61	76	53
HR × FCC, beats × mg/min	C	7999±75	9501±90	100	100	84
	NA	6677±650*	11 737±1163	80	124	57
V_{resp}, μmoles O_2/min·g	C	22,7±2,1	33±3,2	100	100	66
	NA	38,7±3,5*	63,5±6,1	169	192	60
V_f, ml/min	C	7±0,6	9,67±1,6	100	100	72
	NA	9,4±1	14,5±1,2	134	161	65
Lactate, μmoles/min·g	C	7,38±1,03*	2,34±0,63	100	100	315
	NA	14,68±2,42	14,73±4,79	194	596	99
Pyruvate, μmoles/min·g	C	0,623±0,103*	0,224±0,107	100	100	282
	NA	0,777±0,143	0,382±0,36	173	229	203
CPK, μmoles/min·g	C	0,0076±0,01	0,0102±0,02	100	100	74
	NA	0,021±0,02	0,0099±0,001	400	141	212
Lactate/pyruvate	C	118	104	100	100	113
	NA	189	365	159	350	51
HR × FCC/V_{resp}	C	352	287	100	100	122
	NA	172	185	49	64	93

Legend. *) Differences between LR and HR significant at $p < 0.05$ level; C) control (initial state).

nary blood flow rate (V_f, ml/min) was measured. Concentrations of lactate [7] and pyruvate [8] and of creatine phosphokinase (CPK) [9] were determined in the perfusion fluid flowing from the heart.

EXPERIMENTAL RESULTS

The functional-metabolic properties of the myocardium of HR and LR rats different significantly. After a 30-40-min period of postextirpation recovery HR of the myocardium of the LR rats was 15% higher but FCC was 34% lower than in the myocardium of the HR rats (Table 1). As a result the tension developed by the myocardium (HR × FCC) of the LR rats was less (by 17%) than in the myocardium of the HR rats. Since the rate of oxygen consumption in the myocardium of LR rats also was significantly lower (by 34%) than in the HR myocardium, but the rate of lactate formation, on the other hand, was higher (Table 1), it will be evident that the ratio of aerobic to anaerobic processes in the myocardium of the two types of animals was unequal: in the LR myocardium the contribution of glycolysis was significantly greater than in the HR myocardium. This is shown also by calculations of the lactate/pyruvate ratio (Table 1). Nevertheless, the content of ATP and CP in the myocardium of the HR and LR rats was indistinguishable (14.6 and 15.6 μmoles/g dry weight and 20.1 and 19.8 μmoles/g respectively).

Under the influence of noradrenalin (NA, 10^{-8}-10^{-5} M) a dose-dependent change took place in all the parameters studied, and which was abolished by propranolol. Consequently, NA activated myocardial β-adrenoreceptors. Under its influence HR increased virtually without a lag period, and after 5 min it had reached higher values than before exposure to it. In the myocardium of HR rats this increase (Table 1) developed more rapidly and was relatively more marked than in the LR myocardium (42 and 26% respectively). FCC, on the other hand, decreased significantly under the influence of NA, and the decrease was greater in the LR myocardium than in HR (by 39 and 24% respectively). Ultimately the value of HR × FCC fell under the influence of NA by 20% in the LR myocardium, whereas in the HR myocardium, on the contrary, it increased by 24% and became almost twice as high as in LR.

TABLE 2. Changes Under Influence of Noradrenalin (NA) in Amytal- and Malonate-Sensitive Components of Functional Metabolic Preparations in Myocardium of HR and LR Rats (in percent of initial value)

Parameters	Substance to which exposed	LR, %		HR, %	
		control	NA	control	NA
FCC	Amytal	−62	−28	−44	−17
	Malonate	−25	−40	−49	+23
HR	Amytal	−58	−16	−30	+7
	Malonate	+5	−16	+10	+3
HR × FCC	Amytal	−86	−30	−61	−6
	Malonate	−21	−52	−44	+2
V_f	Amytal	−11	+12	+8	+4
	Malonate	−9	−10	−21	+26
V_{resp}	Amytal	−50	+3	−37	+6
	Malonate	−7	−22	−25	+29

In both cases an increase in the intensity of lactate and pyruvate formation and an increase in the lactate/pyruvate ratio were observed, evidence of intensification of glycolysis, which is known to be activated through the cAMP system by adrenoreceptor agonists. Intensification of this process in the myocardium of the HR rats was relatively greater, but considering that the original absolute values of the lactate concentration in this case were lower than in HR myocardium, in the presence of NA it was equalized. Nevertheless, myocardial respiration also was increased by NA, and in the myocardium of HR rats it was, moreover, greater than in LR, and the differences in the rate of oxygen consumption in these two cases reached double figures (Table 1). Thus under the influence of NA marked aerobization of the process took place, more especially in the myocardium of HR rats. Nevertheless, dependence of the contractile function on oxygen was reduced by half in the presence of NA in the myocardium of LR rats, and by one-third in the HR myocardium. There was thus a shift in the ratio of glycolysis to aerobic oxidation in favor of the former. Consequently, activation of myocardial oxygen consumption under the influence of NA largely reflects intensification of free oxidation processes, and not changes in the oxidative phosphorylation system.

This conclusion is supported by experiments with amytal ($5 \cdot 10^{-4}$ M) and malonate ($2 \cdot 10^{-2}$ M), inhibitors of the substrate region of the respiratory chain. We know that in normal HR and LR animals sensitivity of respiration and FCC of the myocardium to these substances differs: for amytal it is greater in LR rats, indicating predominance in this case of the NAD-dependent pathway of oxidation of energy-yielding substrates, whereas for malonate it is greater in HR rats, evidence of predominance of the succinate-dependent pathway of oxidation in the heart of these animals [1, 2]. However, in the presence of NA relative sensitivity to amytal – a nonspecific inhibitor of the first enzyme complex of the respiratory chain, in the myocardium of the two types of animals in most cases either falls or disappears virtually completely (Table 2), evidence of limitation of oxidation of NAD-dependent substrates. Sensitivity of the measured parameters to malonate, a specific inhibitor of succinate dehydrogenase, is reduced only in the myocardium of the HR rats. Thus the increase in oxygen consumption in the HR myocardium in the presence of NA reflects either oxidation in the respiratory chain (for example, through NADH-oxidase of the outer mitochondrial membrane) or intensification of free oxidation, or intensification of both processes at the same time. By contrast, in the LR myocardium the malonate-sensitive component of the measured parameters is increased (Table 2). Consequently, in this case activation of respiration is linked also with intensification of the succinate oxidase pathway of oxidation. Thus NA influences aerobic processes in the myocardium of HR and LR rats differently. The ATP content in the myocardium of HR and LR rats (14.0 and 14.6 μmoles/g dry weight) was not significantly changed, whereas the creatine phosphate concentration was virtually unchanged (23.1 and 20.8 μmoles/g dry weight respectively).

It has to be pointed out that NA also influences vasodilatation. In its presence V_f is intensified, and this is expressed more in the HR myocardium (Table 1). Considering that the vasodilator function is largely determined by release of adenosine as a result of activation of the adrenoreceptors by catecholamines, it can be concluded that the myocardium of HR rats is under stronger control of the sympathetic nervous system than the LR myocardium. Finally, NA modifies the permeability of the cardiomyocyte plasmalemma, which was estimated by the release of CPK into the perfusion fluid. The initial content of CPK in the perfusion fluid was the same in the two groups of animals. However, under the influence of NA, phasic changes took place: an initial rapid decrease in its outflow, reflecting a decrease in membrane permeability, followed by a second rise (greater in the LR than the HR myocardium), evidence of labelization of the membranes (Table 1).

The results not only confirm the earlier facts that the functional-metabolic parameters of HR and LR rats differ, but they are also evidence that myocardial adrenoreceptors are involved in the regulation of oxidative metabolism, correlating with individual resistance of the heart and of the body as a whole to hypoxia.

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