EFFECT OF PARASYMPATHETIC DENERVATION ON OXIDATIVE PHOSPHORYLATION AND ADENOSINE-TRIPHOSPHATASE ACTIVITY OF MITOCHONDRIA IN THE LEFT AND RIGHT VENTRICLES OF DOGS

L. V. Stoida

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Division of the left or right vago-sympathetic nerve in the neck in dogs below the ganglion nodosum leads after 3 days to a decrease in the rate of respiration and phosphorylation in the mitochondria of the left and right ventricles when pyruvate, succinate, and α-ketoglutarate are used as oxidation substrates. The Mg^{++}-dependent ATPase activity of the mitochondria of the left and right ventricles is greatly increased and the stimulant effect of 2,4-dinitrophenol on Mg^{++}-activated ATPase activity is abolished. Thirty days after the operation the rate of respiration and phosphorylation in the mitochondria during oxidation of pyruvate and α-ketoglutarate still remains low, while during oxidation of succinate it shows some degree of normalization. The increase in Mg^{++}-dependent ATPase activity and decrease in the stimulant effect of 2,4-dinitrophenol are less marked.

Removal of the trophic influence of the autonomic nervous system causes severe degenerative changes in the heart [7] and leads to a disturbance of the mechanisms which regulate the myocardial metabolism. Changes in the protein, nucleic acid, and energy metabolism of the myocardium after parasympathetic denervation have been studied in some detail, with allowance for the specific functions of its different parts [3, 10, 5, 8, 9], but too little attention has been paid to the content of oxidative enzymes and to the processes of energy formation in this state. The facts described in the literature on this subject are few in number and they relate mainly to the tissues of the whole heart [1, 10, 6].

In this investigation the effect of parasympathetic denervation was studied on the formation of high-energy phosphorus compounds and on the ATPase activity on the mitochondria of different parts of the dog myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on 33 dogs weighing 16-18 kg. The vago-sympathetic nerve in 24 dogs was divided in the neck below the ganglion nodosum. Three or 30 days after the operation the heart was removed under thiopental-barbital anesthesia (25 mg/kg) and artifical respiration and perfused in ice-cold 0.15 M KCl solution. The mitochondria were isolated by differential centrifugation in 0.32 M sucrose solution with 0.01M EDTA (pH 7.4) and suspended in 0.25 sucrose solution in 0.01 M Tris buffer (pH 7.40). The oxygen intake of the mitochondria was measured by a polarographic method with a stationary platinum electrode in constant temperature cells at 26°C in buffer solution [2]. The exogenous oxidation substrates were: α-ketoglutarate (with the addition of malonate), succinate, pyruvate (plus malate) in a concentration of 10 μ moles

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TABLE 1. Effect of Division of Vago-Sympathetic Nerves on Respiration (in μatoms O_2 per sec per g protein) and on Oxidative Phosphorylation in Mitochondria of Different Parts of the Myocardium in Dogs (M±m)

Substrate	Index	Normal		Division of left vago-sympathetic nerve				Division of right vago-sympathetic nerve			
		3 days		3 days		30 days		3 days		30 days	
		ventricles						heart			
		L	R	L	R	L	R	L	R	L	R
Pyrovate	V_3	2,70±0,1	2,6±0,1	1,72±0,09*	1,54±0,12*	2,2±0,10*	2,0±0,16*	1,7±0,15*	1,4±0,14*	1,88±0,11*	1,7±0,08*
	V_4	1,0±0,06	1,0±0,06	0,78±0,08*	0,75±0,08*	0,78±0,04*	0,76±0,07*	0,85±0,08*	0,8±0,12*	0,73±0,07*	0,66±0,04*
	RC	2,7±0,15	2,6±0,13	2,3±0,17	2,0±0,17	2,9±0,23	2,6±0,45	2,0±0,1*	1,7±0,16*	2,63±0,1	2,7±0,03
	ADP/O	2,7±0,09	2,7±0,1	2,2±0,08*	2,2±0,12	2,7±0,08	2,3±0,05*	2,7±0,12	2,8±0,2	2,6±0,2	2,6±0,2
Succinate	V_3	3,3±0,23	3,1±0,21	2,2±0,28*	1,6±0,11	2,7±0,1*	2,9±0,1	1,2±0,33*	1,25±0,25*	2,97±0,8	2,83±0,3
	V_4	1,7±0,13	1,6±0,07	1,25±0,17*	1,12±0,1 -	1,7±0,1	1,75±0,10	0,78±0,2*	0,54±0,06*	1,64±0,29	1,8±0,18*
	RC	2,0±0,06	2,0±0,11	1,75±0,09*	1,50±0,08*	1,66±0,1*	1,70±0,18	1,5±0,25	2,14±0,46	1,83±0,2	1,6±0,06*
	ADP/O	1,9±0,07	1,9±0,06	1,9±0,08	1,8±0,1	1,9±0,12	1,9±0,1				
α-Keto-glutarate	V_3	2,6±0,2	2,1±0,2	1,3±0,13*	1,1±0,2*	1,90±0,2*	1,66±0,2	0,9±0,07*	1,12±0,13*	1,87±0,14	2,02±0,17
	V_4	0,8±0,10	0,78±0,16	0,57±0,08	0,48±0,03	0,58±0,07	0,64±0,15	0,47±0,06*	0,5±0,15	0,6±0,05	0,69±0,2
	RC	3,3±0,34	2,7±0,23	2,4±0,23*	2,25±0,28	3,07±0,16	2,7±0,6	1,9±0,2*	2,24±0,2	3,06±0,3	3,00±0,3
	ADP/O	3,6±0,09	3,6±0,1	2,6±0,13*	2,3±0,25*	3,1±0,07*	3,1±0,1	2,6±0,5*	1,8±	3,2±0,12*	3,3±0,1*
Number of dogs . .		9		5		7		5		7	

Legend: V_3 rate of O_2 intake by mitochondria in state of active respiration; V_4 rate of O_2 intake by mitochondria in the control state.

* – $P < 0.05$;

RC – respiratory control (after Chance and Williams).

per 1-ml sample. ADP was added in a quantity of 0.24 μ mole. The protein concentration in the mitochondria was 1.0-1.5 mg per sample. Parallel tests were carried out on four samples. The rate of respiration was expressed in microatoms O_2 per second per gram mitochrondrial protein. The mitochrondrial ATPase activity was calculated from the increase in inorganic phosphorus during hydrolysis of ATP after incubation for 10 min at 26°C in samples containing 600 μmoles KCl, 40 μmoles $MgCl_2 \cdot 6H_2O$, 10 μmoles ATP-Na, and 0.25 sucrose (pH 7.4). The mitochondrial protein concentration was 0.3-0.5 mg per sample and the final concentration of added 2,4-dinitrophenol was 3×10^{-4} M. The ATPase activity was expressed in microatoms phosphorus per mg mitochondrial protein per hour. The concentrations of mitochondrial protein and phosphorus were estimated by Lowry's method [11, 12]. The experimental results were subjected to statistical analysis by Student's method [4].

EXPERIMENTAL RESULTS

Under normal conditions no significant differences were found in the intensity of respiration and phosphorylation between the mitochondria of the left and right ventricles in dogs (Table 1). Division of both left and the right vago-sympathetic nerve led after 3 days to a decrease in the rates of oxidation and phosphorylation in the mitochondria of both ventricles on all oxidation substrates (Table1). The coupling of respiration with phosphorylation was disturbed. The ADP/O ration was significantly below normal only during oxidation of α-ketoglutarate. These changes were more marked after division of the right vagus nerve.

The use of NAD-dependent substrates and of succinate revealed differences in the oxidative and phosphorylating activity of the mitochondria isolated from the left and right ventricles after partial parasympathetic denervation.

Thirty days after division of the left or right vagus nerve the rat of respiration and of respiration coupled with phosphorylation was still below normal when pyruvate and α-ketoglutarate were used as the oxidation substrates. When succinate was used these processes had returned to normal.

The Mg^{++}-dependent ATPase activity of the mitochondria under normal conditions was equal in the left and right ventricles: 33.6 and 32.2 μatoms P/mg/h respectively. Addition of 2,4-dinitrophenol (2,4-DNP) to the incubation medium stimulated the rate of ATP hydrolysis by 100%.

Division of either the right or the left vagus nerve caused a significant increase in Mg^{++}-dependent ATPase activity 3 days after the operation (by 60% in the mitochondria of the left ventricle and by 40% in the right). The percentage stimulation of Mg^{++}-dependent ATPase activity by 2,4-DNP fell. These changes were less marked on the 30th day.

The decrease in the rate of respiration and of the phosphorylation coupled with it, the increase in Mg^{++}-dependent ATPase activity, and the decrease in the stimulant effect of 2,4-DNP in the mitochondria of the left and right ventricles after partial parasympathetic denervation presumably indicate certain destructive changes in the mitochondria and a decrease in activity of the oxidative enzymes. This conclusion is in agreement with various experimental results [1, 10, 6] showing a decrease in enzymic activity and in the oxygen utilization in the heart tissue.

Removal of central parasympathetic nervous influences from the heart also leads to some disturbance of the utilization of the energy of high-energy compounds by the myocardium. The writers have shown previously [9] that division of the left or right vagosympathetic trunk causes an increase in the creatine phosphate concentration in the tissue of the left and right ventricles by 50-58%. While the ATP content remains constant, the ADP concentration rises to 40%, the AMP to 74%, and the ADP/ATP ration increases.

Partial parasympathetic denervation of the heart in the early stages thus leads to a decrease in the rate of respiration and phosphorylation in mitochondria isolated from the myocardium of the left and right ventricles in dogs, an increase in Mg^{++}-dependent ATPase activity of the mitochondria, and a decrease in the stimulating effect of 2,4-DNP on the ATPase activity. In the late stages of denervation these processes are partly restored to normal, although the utilization of the energy of high-energy compounds by the myocardium remains disturbed.

LITERATURE CITED

1. V.A. Govyrin, in: The Evolution of Function [in Russian], Moscow-Leningrad (1964), p. 241
2. A.M. Zubovskaya, N.O. Stepanyan, and S.E. Severin, Vopr. Med., Khimii, No. 5, 533 (1968).
3. L.A. Kopteva and N.M. Dmitrieva, Byull. Éksperim. Biol. i Med., No. 4, 47 (1966).
4. P.F. Rokitsskii, Fundamentals of Variance Analysis [in Russian], Minsk (1961).

5. V.M. Rubel' and G.V. Chernysheva, Problems in Neurotrophic Mechanisms in the Theory and Practice of Medicine [in Russian], Moscow (1963), p.286
6. E.S. Troshanova, Ukr. Biokhim. Zh., No.3, 312 (1952).
7. L.A. Chernova, Arkh. Anat., No.7, 13 (1960).
8. G.V. Chernysheva, Byull. Eksperim. Biol. i Med., No. 7, 52 (1966).
9. G.V. Chernysheva and L.V. Stoida, Byull. Éksperim. Biol. i Med., No. 2, 53 (1969).
10. L. Komadel, E. Barta, and M. Kokavec, Physiological Enlargement of the Heart [in Russian], Bratislava (1968), p.142
11. O.H. Lowry and J. A. Lopes, J. Biol. Chem., 162, 421 (1946).
12. O.H. Lowry et al., J. Biol. Chem., 193, 265 (1951).