BIOCHEMISTRY AND BIOPHYSICS

STUDY OF METABOLISM OF PHOSPHORUS COMPOUNDS IN THE HEART, USING RADIOACTIVE PHOSPHORUS

PART II. EFFECT OF PAVLOV'S "REINFORCING NERVE" ON THE VELOCITY OF FORMATION OF PHOSPHORUS COMPOUNDS IN THE DOG'S HEART

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I. P. Pavlov [1] expressed the view that chemical processes in the tissues are regulated by special nerve fibers, and in the heart, in particular, by the "reinforcing nerve", discovered by him. The nature of the chemical substrate of the action of this nerve has not, up to the present, been studied.

In attempting to discover the point at which the action of the reinforcing nerve is exerted, we took as our starting point the important role of the macroenergetic phosphorus compounds in contraction of the heart muscle. It seemed probable that the action of the reinforcing nerve on the heart is effected by intensification of the metabolism of such substances as creatinephosphate and adenosinetriphosphate.

Of the available methods of biochemical study, only that based on the use of radioactive isotopes indicates the intensity of metabolism of particular substances in the organism. A number of workers have been able, by means of this method, to elucidate the metabolism of creatinephosphate and adenosinetriphosphate in the heart muscle.

Furchgott and Shorr [4] have shown that the renewal of all the phosphorus of phosphocreatine and of the terminal phosphorus of adenosinetriphosphate is effected in 30 minutes at 37° in slices of heart muscle. These authors showed, by separate studies of the activity of extra- and intracellular inorganic phosphate fractions, that it is the latter which is the direct donor of phosphate to the phosphorus-organic compounds of the tissues. After 30-90 minutes of incubation of heart slices with P^{32} the same activity is found for intracellular inorganic phosphate P, phosphocreatine P, and adenosinetriphosphate terminal P.

Sacks and Altshuler's [5] results for cat heart in situ are of even greater interest. These authors showed that the rate of incorporation of inorganic plasma phosphate into A.T.P. and creatinephosphate was 20 times greater for heart muscle than for skeletal muscle.

The P^{32} content of heart muscle A.T.P. and creatinephosphate rises to a maximum 2 hours after its subcutaneous introduction, falling thereafter.

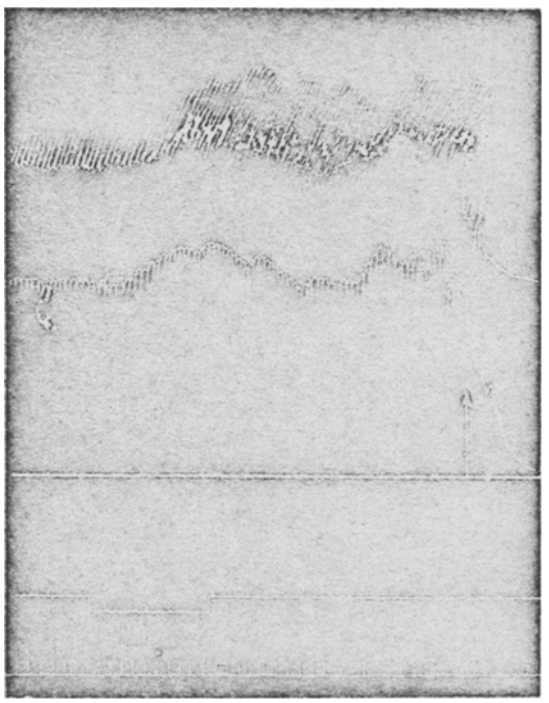
We have found no references in the literature to nervous regulation of the metabolism of energy-rich compounds of the heart muscle.

The present paper describes the results of a study of the effect of the reinforcing nerve of Pavlov on inorganic phosphate, creatinephosphate, and A.T.P. metabolism in the heart muscle, using the radioactive phosphorus isotope method.

EXPERIMENTAL METHODS

The experiments were performed on dogs weighing 10-12 kg. The animals were fasted on the day of the experiment.

The thorax was opened under morphine-barbamyl narcosis, and artificial respiration was instituted.



Change in blood pressure and amplitude of heart beat following stimulation of the "reinforcing nerve". Explanation of tracings (from above down): blood pressure (elastic manometer); blood pressure (mercury manometer); zero line; stimulation signal; time marker. The arrow indicates moment of cutting off of the apex of the heart.

The right vagus nerve was severed in the neck, while registering arterial blood pressure in the left subclavian artery. The vagus was exposed, including its cardiac branch ("Pavlov's nerve") below the inferior cervical ganglion. In order to find the strength of stimulus needed to give a clear-cut strengthening of the heartbeat, we performed test stimulations of the nerve, using a Du Bois Reymond induction coil.

After a 15-minute interval an injection of $Na_2HP^{32}O_4$ was made into the femoral vein, at a dosage of 15 million impulses per kg body weight. The apex of the heart was cut off, precisely at predetermined times. The same was done to the control animals during normal functioning of the heart, whereas the specimen was taken from the experimental animals at the moment when the effect of stimulation of the cardiac nerve became evident (blood pressure was recorded at this moment, and an electrocardiogram was taken).

We endeavored to maintain the conditions during the physiological part of the experiments as standard as possible. The experiments were conducted according to a rigidly laid-down schedule, with the same sequence and duration of manipulations, and with systematic registration of blood pressure and of electrocardiograms (in order to check the functional state of the heart).

The specimen of heart muscle was immediately plunged into liquid nitrogen and ground to a fine powder, to which were added 2 volumes of cooled 5% trichloroacetic acid, in order to precipitate protein. The inorganic phosphate, creatinephosphate, and adenosinetriphosphate contents of the filtrate were determined.

Inorganic phosphate was rapidly precipitated from weakly alkaline solution by means of barium acetate. The precipitate was dissolved in hydrochloric acid, and barium was removed by precipitation with sodium sulfate. The phosphorus content of the filtrate was determined after adjusting the concentration of acid to normal.

Phosphocreatine was determined in the barium-soluble portion of the trichloroacetic acid filtrate. Barium was removed as sulfate, and the concentration of acid was adjusted to normal. The phosphorus content was determined after 30 minutes, required for hydrolysis of phosphocreatine to give inorganic phosphate.

The readily hydrolyzable phosphorus of adenosinetriphosphate was determined in a second portion of filtrate. A.T.P. was precipitated with mercuric acetate, and phosphorus was determined before and after hydrolysis for 10 minutes on a boiling water bath.

Parallel with the chemical analyses we determined the activity of each of the fractions. With this object, we added carrier to the mineralized phosphate solution from each fraction, and precipitated with magnesia mixture. The precipitate was dissolved in hydrochloric acid, and an aliquot was placed in a glass planchette and dried. Activity was determined using a "B" equipment with a torsion counter.

EXPERIMENTAL RESULTS

The functional effect of stimulation of the "reinforcing nerve" has been described in detail in one of our earlier papers [2]. It amounts, basically, to raising of the blood pressure and of the amplitude of the heartbeat, without change in rhythm in most cases (see figure).

It was essential, in order to study the effect of stimulating the reinforcing nerve on incorporation of P^{32} into phosphocreatine and adenosinetriphosphoric acid of heart muscle, to find times of sampling during the ascending part of the curve representing uptake of P^{32} by the fractions studied.

Content and Specific Activity (Calculated on the Basis of Introduction of 10^6 Impulses per Minute Per Kg Body Weight) of Phosphorus Fractions from Dog Heart Muscle, in the Normal State and After Stimulation of the "Reinforcing Nerve"*

No. of experiment	Date of experiment	Sex of dog	Weight of dog (g)	Phosphorus content (mg-%)			Activity of the whole heart (as % of that introduced)	Specific activity of phosphorus		
				inorganic phosphate	creatine-phosphate	adenosine triphos-phate		inorganic phosphate	creatine-phosphate	adenosine triphos-phate
Normal										
60	(1953)									
	4/24	Female	10 350	35.8	5.3	38.0	1.3	3 184	—	1 006
69	7/13	Male	13 500	35.2	12.5	30.8	2.2	3 014	792	812
67	7/8	Female	9 500	30.3	7.8	28.9	1.3	3 835	1 564	1 000
51	3/20	"	11 000	36.0	6.0	22.0	—	1 403	963	—
70	7/15	Male	13 500	28.8	6.2	27.9	1.2	2 611	984	473
59	4/20	Female	10 300	37.0	8.4	36.3	0.8	2 260	786	132
63	6/17	"	12 200	34.9	4.9	31.3	1.0	2 521	—	1 250
57	4/10	"	12 600	38.8	9.6	27.6	0.9	1 809	843	485
Mean				34.6	7.6	30.3	1.2	2 580	988	737
With stimulation of the "reinforcing nerve"										
55	4/1	Female	14 500	39.3	12.4	36.3	2.2	4 765	2 104	1 333
56	4/2	"	9 000	17.9	11.1	22.1	2.9	7 195	1 730	3 574
51	3/28	"	11 300	30.8	18.5	16.3	—	4 912	1 151	—
62	4/15	Male	13 000	35.3	5.8	40.0	2.1	3 626	2 552	925
66	7/6	Female	11 500	38.8	5.6	39.5	2.4	3 600	1 910	1 111
65	6/24	"	11 500	28.5	6.6	24.8	1.6	3 600	1 833	846
64	6/19	Male	12 600	33.9	2.3	30.1	1.5	3 256	3 700	442
68	7/10	"	10 200	43.5	14.4	27.2	0.9	1 600	896	820
Mean				33.5	9.6	29.5	1.9	4 069	1 984	1 293
As percentage of normal					126		158	158	201	175

* The heart was removed 15 minutes after introduction of P^{32}.

We showed in our previous communication [3] that uptake of phosphorus by the heart from the blood flowing through it proceeds with very high intensity. We found in preliminary experiments that during 15 minutes after introduction of P^{32} into the blood stream its rate of uptake by creatinephosphate exceeds that of its elimination, while towards the end of this period these rates are equalized for adenosinetriphosphate. By sampling at the end of 15 minutes, we were enabled to follow creatinephosphate phosphorus metabolism on the rising branch of the curve, and A.T.P. phosphorus metabolism in its steady state. We performed 8 control experiments, and 8 experiments with stimulation of the "reinforcing nerve", within 15 minutes of injection of P^{32}.

It is evident from the table that stimulation of the reinforcing nerve does not significantly affect the inorganic phosphate, creatinephosphate, and adenosinetriphosphate contents of heart muscle; there is possibly a slight increase in creatinephosphate content.

The specific activity of all fractions is raised after stimulation of the reinforcing nerve; the levels for inorganic phosphate, creatinephosphate, and adenosinetriphosphate are, respectively, 158, 201, and 175% of the normal ones.

It may be concluded from our results that stimulation of the "reinforcing nerve" causes an increase in the rate of turnover of phosphorus contained in inorganic phosphate, creatinephosphate, and adenosinetriphosphate of heart muscle. This property of intensifying the process of renewal of inorganic phosphate and energy-rich compounds of heart muscle appears to be connected with the intimate chemical mechanism of action of the reinforcing nerve, discovered by I. P. Pavlov.

LITERATURE CITED

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[3] M. E. Raiskina, Byull. exptl. Biol. i Med. 41, No. 3, pp. 46-49 (1956) (T.p. 241)**

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[5] J. Sacks and C. H. Altshuler, Am. J. Physiol. 137, p. 750-760 (1942).

*In Russian.

**T.p. = C. B. Translation pagination.