Brit. J. Pharmacol. (1957), 12, 270.

THE EFFECT OF DINITROPHENOL, HYPOXAEMIA AND ISCHAEMIA ON THE PHOSPHORUS COMPOUNDS OF THE DOG HEART

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(RECEIVED NOVEMBER 9, 1956)

The results reported in this paper indicate that dinitrophenol acts directly on the isolated heart, increasing its metabolic rate. It also produces heart failure associated with a low phosphocreatine content of the muscle but with no change in adenosine triphosphate, which may or may not be due to a relative hypoxia of the cardiac tissue. Experimental arterial hypoxaemia, if severe, produces a similar picture of heart failure with a decrease in phosphocreatine and no change in adenosine triphosphate. Ligation of the coronary arteries results in disappearance of the major part of the phosphocreatine within a few minutes regardless of whether or not ventricular fibrillation ensues; the adenosine triphosphate remains unchanged.

2:4-Dinitrophenol increases oxidative metabolism in animals several-fold by direct cellular action (Tainter and Cutting, 1933). It is also known to uncouple phosphorylation and oxidation in tissue homogenates or particulate systems derived therefrom (Loomis and Lipmann, 1948). Hence, if dinitrophenol were added to a well-oxygenated heart-lung preparation, one would expect to observe a failure of the heart. This failure would be due to lack of *production* of high-energy phosphate bonds, instead of lack of *utilization* of such bonds as is the case in heart failure due to barbiturates (Wollenberger, 1947; Fawaz and Hawa, 1953). This study was undertaken to determine to what extent observations made on homogenates and simpler systems can be applied to organs in activity. Since dinitrophenol may produce relative hypoxia by increasing metabolism, the effects of arterial hypoxaemia and ischaemia on the phosphorus compounds of the heart were also studied.

METHODS

Pentobarbitone anaesthesia was used in all animals. Heart-lung preparations were made by the conventional procedure except that the blood donors were anaesthetized with chloroform.

Detailed information for preparing samples from the left ventricle and for estimating the phosphorus compounds has been described previously (Fawaz and Hawa, 1953). In the present work, the labile nucleotide phosphorus was determined by hydrolysing the trichloracetic acid filtrate in N-HCl for 7 instead of 10 min. Experiments with pure crystalline adenosine triphosphate (ATP) showed that this was a sufficient period for 2/3 of the ATP phosphorus to be liberated. Our values are therefore slightly lower than before. In several control experiments and in a dinitrophenol experiment we compared the results obtained by this method with the paper chromatographic method for ATP determination. Nucleotides were precipitated from the trichloracetic acid filtrates with mercuric acetate (Kerr, 1940). After removal of the mercury by H_2S and evaporation to a small volume, the nucleotides were chromatographed on filter paper by the method of Magasanik, Vischer, Doniger, Elson, and Chargaff (1950). It was found that at least 90% of the 7 min. value, as determined on the trichloracetic acid filtrate, was accounted for by true ATP.

The phosphorus values in this paper are expressed not as mg./100 g. wet tissue but as % of the total acid-soluble phosphorus. Since it so happens that the total acid-soluble phosphorus is about 100 mg./100 g. the two methods of calculation give similar figures.

With the exception of Experiments 1 to 7 (Table I), the lungs in all dinitrophenol experiments were ventilated with pure oxygen. In addition, oxygen was bubbled through the venous reservoir by means of a Pyrex glass dispersion tube with fritted glass cylinder, foaming being prevented by the use of Dow Corning Antifoam A. The oxygen content of the arterial and coronary sinus blood was determined by the Van Slyke method, samples being taken at the beginning of the experiment and again just before the heart specimen was removed. Coronary sinus blood was

collected by means of a Morawitz cannula which also served to measure sinus flow. The cuff of the Morawitz cannula was inflated only when samples were withdrawn or measurements taken, because continued inflation produced widespread oedema and haemorrhagic necrosis of the left ventricle in some preparations. Hypoxaemia in the heart-lung preparation was produced by gradually diminishing the stroke-volume of the respiration pump. Ischaemia of the left apical ventricular area was produced in the intact animal by ligating the anterior descending and circumflex branches of the left coronary artery, leaving the septal branch intact. Ventricular fibrillation occurred a few min. after occlusion in 5 out of 7 experiments.

RESULTS

Dinitrophenol greatly increased the oxygen consumption of the heart. Although blood entering the heart from the venous reservoir was well oxygenated and the lungs were ventilated with oxygen, the systemic blood leaving the heart was dark and had a lower oxygen content (Table I). Measurements of coronary sinus flow and arteriovenous oxygen difference also showed increased oxygen utilization after dinitrophenol, but we consider this method of measuring oxygen consumption to be unreliable. The work of Katz, Jochim, and Weinstein (1938) and of Moe and Vischer (1940) shows that the coronary sinus flow does not represent a constant fraction of total coronary flow, nor is the oxygen content of the coronary sinus blood identical with that of the Thebesian blood. From our own observations it appears unlikely that the coronary sinus flow itself is accurately measured by the Morawitz cannula in the dog. When the heart was opened at the end of each experiment to make sure that the cannula was still in place, we discovered that in most dogs there were openings of varying magnitude in the walls of the sinus which would be blocked by inflating the rubber cuff of the cannula. The real purpose of the Morawitz cannula in our experiments was, therefore, to obtain samples of coronary sinus blood for oxygen determination; the estimates of coronary sinus flow and oxygen consumption have only qualitative significance.

Infusion of dinitrophenol sodium at the rate of 6 mg./min. usually produced no signs of failure during the first 10 to 15 min. Then there was a rise in venous pressure, usually not exceeding 2 cm. of water, with a decrease in systemic output but with no change of arterial blood pressure. Later, a point was reached when the blood pressure began to fall rapidly and cardiac arrest occurred in a few minutes. The decrease in systemic output does not necessarily reflect a fall in total left ventricular output because it might be compensated by a corresponding increase in coronary flow (Fawaz and Tutunji, 1957).

TABLE I

EFFECT OF DINITROPHENOL SODIUM ON THE ISOLATED DOG HEART

Phosphorus compounds are expressed as % of the total acid-soluble phosphorus.

Dinitrophenol-Sodium Infused (mg.)	Duration of Experiment (min.)	Systemic Output (ml./min.)		Coronary Sinus Flow (ml./min.)		O_2 Content of Arterial Blood (Vol. %)		O_2 Content of Coronary Sinus Blood (Vol. %)		Change in O_2 Consumption	Phospho-creatine-P	Inorganic-P	Labile Nucleotide-P
		Begin.	End	Begin.	End	Begin.	End	Begin.	End				
166	42	760	440								0	31·0	33·6
120	31	600	380								0	33·8	32·8
120	31	720	220								0	31·2	29·4
60	48	940	560								6·6	26·3	34·8
30	40	770	80								2·3	26·6	29·0
156	95	880	480								2·6	32·6	28·9
150	34	590	320								5·5	28·4	33·5
150	44	840	190								0	29·2	35·2
150	31	770	300								3·7	28·6	32·6
100	133	790	250								8·9	24·3	31·2
150	31	680	500	39	220	16·7	9·4	6·0	4·3	+170%	0	29·0	32·2
150	29	740	300	31	65	16·7	12·0	9·4	4·3	+120%	6·0	28·6	33·6
150	41	550	290	46	110	19·7	13·7	6·9	5·1	+163%	5·8	30·6	26·6
120	37	600	140	17	—	17·6	8·6	6·0	2·6		2·2	35·6	27·0
150	40	880	0	46	190	17·7	12·1	9·9	8·6	+85%	0	27·7	35·7
150	30	760	290	20	47	16·5	11·8	5·7	5·2	+44%	0	35·3	27·9
150	29	640	500	24	200	15·9	8·9	4·4	1·9	+407%	0	39·7	26·5
150	34	740	360	13	54	27·4	22·9	8·0	4·0	+305%	4·3	33·3	26·4
150	34	800	420	18	49	26·5	20·3	6·2	3·5	+126%	0	33·7	26·8
120	36	670	380	47	173	16·7	8·8	5·3	4·4	+42%	4·8	34·1	17·1
150	36	990	120	30	—	25·1	16·7	6·2	7·0		5·4	30·8	28·2
150	32	860	580	34	—	20·5	15·1	8·0	2·7		0	36·0	28·4
120	33	800	540	24	113	21·8	16·0	6·2	5·4	+220%	0	32·2	25·3
										Average ± S.E.	2·5±0·6	31·2±0·8	29·7±0·9

TABLE II

EFFECT OF HYPOXAEMIA ON THE ISOLATED DOG HEART

Phosphorus compounds are expressed as % of the total acid-soluble phosphorus.

Duration of Hypoxaemia (min.)	Systemic Output (ml./min.)		Coronary Sinus Flow (ml./min.)		O_2 Content of Arterial Blood (Vol.%)		O_2 Content Coronary Sinus Blood (Vol.%)		Change in O_2 Consumption	Phospho-creatine-P	Inorganic-P	Labile Nucleotide-P
	Begin.	End	Begin.	End	Begin.	End	Begin.	End				
54	650	320	28	108	17·5	1·8	6·8	0·6	−57%	0	35·7	30·2
70	900	530	30	48	21·0	10·2	4·0	4·2	−44%	20·7	17·6	36·4
55	820	440	53	105	13·4	7·6	4·9	2·3	+24%	15·5	24·6	27·0
51	800	690	26	38	20·0	14·7	4·0	4·9	−11%	21·4	13·3	32·6
62	560	390	24	36	14·7	9·3	4·9	4·9	−32%	22·5	15·5	33·2
50	640	380	173	149	12·1	2·2	5·0	1·1	−87%	6·3	28·1	26·7
40	600	300	48	216	10·5	1·8	3·0	0·7	−34%	5·6	31·3	28·0
45	750	630	26	50	12·9	7·3	3·8	3·4	−18%	21·7	18·5	28·2
45	612	570	68	108			5·0	3·4		12·0	24·4	31·0
22	468	468	36	62			9·3	4·0		17·8	18·9	31·0

Gradual arterial hypoxaemia produced a similar sudden terminal fall in blood pressure. In the dinitrophenol experiments, the heart specimen was removed just after the sudden onset of failure, and in the hypoxaemia experiments when the coronary sinus blood was judged to be as dark as in the dinitrophenol experiments (Table II).

Dinitrophenol failure is associated with a marked diminution in the phosphocreatine content of heart muscle. The same thing is observed in marked hypoxaemia. The decrease in phosphocreatine in the dinitrophenol-treated heart might therefore be due to relative hypoxia secondary to the increase in metabolism. However, it can be seen from a comparison of Tables I and II that the oxygen content of the coronary sinus blood in the hypoxaemia experiments fell to extremely low levels before the creatine-phosphate reached the low values observed in dinitrophenol experiments with higher oxygen concentrations in the coronary sinus blood. There is an element of uncertainty regarding the adequacy of the control hypoxaemia experiments because the criteria for hypoxia in tissues respiring normally may not be the same as those in tissues with an abnormally high oxidative rate.

Ischaemia caused by ligating the coronary vessels resulted in a disappearance of most of the phosphocreatine within a few minutes, whether or not ventricular fibrillation occurred. This is shown in Table III. Here again the labile ATP phosphorus remained essentially unchanged.

A control series of 9 normal dog hearts (not heart-lung preparations) were analysed for phosphorus compounds during the same period of time in which the above results were obtained. The figures were: phosphocreatine-P, 15.9 ± 0.5; inorganic-P, 19.3 ± 1.4; labile "nucleotide"-P, 31.4 ± 0.9.

TABLE III

EFFECT OF ISCHAEMIA ON THE PHOSPHORUS COMPOUNDS OF DOG HEART IN INTACT OPEN-CHEST ANIMALS

Phosphorus compounds are expressed as % of the total acid-soluble phosphorus.

Duration of Ischaemia (min.)	Interval between Ligation and Vent. Fibrillation (min.)	Phospho-creatine-P	Inorganic-P	Labile Nucleotide-P
2	1	2·0	36·8	26·8
4	—	2·8	33·4	27·8
4	—	2·0	32·6	30·2
2·2	1·8	1·6	32·7	28·7
1·5	1·5	2·3	28·4	35·1
2	1	3·3	35·2	22·5
4	3	3·0	37·8	32·2
Average ± S.E.		2·4 ± 0·2	33·8 ± 1·2	29·0 ± 1·5

This study was supported by a grant from the American Heart Association.

REFERENCES

Fawaz, G., and Hawa, E. S. (1953). *Proc. Soc. exp. Biol., N.Y.*, **84**, 277.

—— and Tutunji, B. (1957). *Brit. J. Pharmacol.*, **12**, 273.

Katz, L. N., Jochim, K., and Weinstein, W. (1938). *Amer. J. Physiol.*, **122**, 252.

Kerr, S. E. (1940). *J. biol. Chem.*, **132**, 147.

Loomis, W. F., and Lipmann, F. (1948). Ibid., **173**, 807.

Magasanik, B., Vischer, E., Doniger, R., Elson, D., and Chargaff, E. (1950). Ibid., **186**, 37.

Moe, G. K., and Vischer, M. B. (1940). *Blood, Heart and Circulation* (*Publ. Amer. Ass. Advanc. Sci.*), 100.

Tainter, M. L., and Cutting, W. C. (1933). *J. Pharmacol.*, **48**, 410.

Wollenberger, A. (1947). *Amer. J. Physiol.*, **150**, 733.