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Effects of catecholamines on rat myocardial metabolism.

I. Influence of catecholamines on energy-rich nucleotides and phosphorylated fraction contents (*).

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Summary. — 1. The influence of catecholamines (adrenaline and noradrenaline) on energy metabolism of the rat myocardium has been studied by incubating slices of this tissue with these hormones and by following the levels of the different phosphorylated fractions and adenylic nucleotides.

2. Similar effects are obtained with both hormones, adrenaline being more effective.

3. Catecholamines decrease significantly the total amount of phosphate while P_i content increases during the first 10 minutes of incubation ; labile and residual phosphate contents increase at the beginning of incubation and decrease to the initial values afterwards.

4. ATP and ADP levels decrease significantly with both hormones ; however, the effect of noradrenalin on the ATP level needs a longer time of incubation. The ATP/ADP ratios decrease after 5 minutes incubation and the total adenylic nucleotide content is severely decreased (35 per cent with adrenalin, after 20 minutes incubation).

5. Similar results have been obtained with other tissues ; these results can explain the decrease of aerobic metabolism we observed under the same conditions.

INTRODUCTION.

Catecholamines — especially adrenaline and noradrenaline — are known as important effectors of physiological and metabolic equilibria in the organism ; this is particularly true concerning the regulation of cardiac muscle activity [1]. On the other hand, several pharmacodynamic products used in cardiovascular therapeutics act, directly or undirectly, through catecholamines [2].

In order to study the biochemical actions of catecholamines in the myocardium, we undertook to follow the possible variations in the levels of phosphorylated fractions in this tissue induced by these hormones, since the metabolic activity of a tissue such as muscle is directly controlled by the level of energy-rich compounds such ADP or ATP. This investigation consists in measuring phosphorylated fractions and nucleotide contents in rat myocardium slices incubated with or without adrenaline or noradrenaline 10^{-5} M.

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MATERIALS AND METHODS.

Incubations.

CFE male rats, 2 to 3 months old, were used for all experiments ; after decapitation, hearts were removed immediately, washed and weighed at 0°C ; then slices — about 150 mg — are incubated in 3 ml Krebs-Ringer bicarbonate, temperature 30°C, shaken continuously, with or without adrenaline or noradrenaline 10^{-5} M ; after 5, 10 or 20 minutes incubation, the myocardium slices are homogenized at 0°C with an Ultra-Turrax, in the presence of 10 p. cent trichloracetic acid. The homogenate is centrifuged 15 minutes at 25000 g at 0°C and the supernatant, which contains free nucleotides, is extracted 4 times with ether at 0°C to remove TCA ; after the last extraction, ether is evaporated.

Adrenaline and noradrenaline were obtained from Fluka.

Separation and estimation of free nucleotides [3].

The free nucleotides are then adsorbed on charcoal columns (Norit S × 30 special) then eluated with 6 ml pyridine 50 p. cent, under pressure

1 atmosphere ; the eluate is evaporated to dryness and the pyridine nucleotide cristals obtained are dissolved in 0.5 ml bidistilled water. A bidimensional chromatography is performed on Whatman n° 1 paper pretreated with EDTA 0.2 p. cent [4], which separates the nucleotides and permits their characterization (fluorescence at 254 nm) ; the first solvant system used is : isobutyric acid - ammonia N 10:6 [5] and the second solvant system is : n-propanol - ammonia - water 6:3:1 [6]. Nucleotides are quantitated after mineralization according to their phosphorus content [7].

Separation and estimation of the phosphorylated fractions.

The technique is essentially as described by Volfin *et al.* [3] and the fractions are defined as follows :

— *Total phosphate :* total acid soluble phosphate.

— *Labile phosphate :* phosphate liberated after spontaneous hydrolysis of very labile esters at room temperature (creatine phosphate mostly).

— *Inorganic phosphate :* intra and extracellular inorganic phosphate.

— *Residual phosphate :* phosphorylated compounds which are neither adsorbed on charcoal nor extracted with isobutanol after hydrolysis at room temperature (mostly phosphorylated intermediates from glycolysis).

RESULTS.

1. *Incubation with adrenaline 10^{-5} M.*

Total phosphate content of myocardial tissue (table I) remains constant for 5 minutes incuba-

TABLE I.

Influence of adrenaline 10^{-5} M on phosphorylated fractions of rat myocardium.

Incubation time	Total phosphate				Inorganic phosphate			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	10	7	6	7	10	7	6	7
$\overline{X} \pm \sigma$	863 ± 21	783 ± 28	740 ± 14	514 ± 16	142 ± 13	311 ± 32	318 ± 21	248 ± 5
F		1,42	0,28	65,24		29	0,001	2,34
F (0,05)		4,54	4,84	4,84		4,54	4,84	4,84
F (0,01)		8,68	9,65	9,65		8,68	9,65	9,65
Significance		—	—	+++		++	—	—

Total phosphate between 0 and 10 min : F = 17,26 ; F(0,05) = 4,60 ; F(0,01) = 8,86 ; S = ++.

Inorganic phosphate : non significant between 5 min and 20 min.

$\overline{X}$ = mean values ; σ = standard error.

Incubation time	Labile phosphate				Residual phosphate			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	10	7	6	7	10	7	6	7
$\overline{X} \pm \sigma$	19 ± 2	35 ± 4	26 ± 4	22 ± 1	242 ± 30	452 ± 23	388 ± 21	241 ± 12
F		14	2,29	1,35		25	1,12	7,10
F (0,05)		4,54	4,84	4,84		4,54	4,84	4,84
F (0,01)		8,68	9,65	9,65		8,68	9,65	9,65
Significance		++	—	—		++	—	+

Labile phosphate between 5 and 20 min : F = 10,4 ; F(0,05) = 4,75 ; F(0,01) = 9,33 ; S = ++.

Results are given in µg P_i/100 g fresh tissue ; the statistical significance is given according to F test (8).

tion, then decreases significantly after 10 minutes, to reach 40 p. cent decrease after 20 minutes incubation. Inorganic phosphate content, on the contrary, increases during the first 5 minutes to keep constant afterwards. Labile phosphate content increase also after 5 minutes, but, after 20 minutes incubation it decreases to reach the starting value. Residual phosphate content follows a similar variation.

Adenylic nucleotides represent about 50 p. cent of total acid-soluble phosphate, 30 p. cent of it corresponding to ATP in agreement with other results [9]. Incubation with adrenaline induces immediately a regular decrease in ATP content, to reach 50 p. cent of the normal value after 20 minutes incubation (table II). Similar variations are given with ADP content which decreases to 60 p. cent after 20 minutes incubation. With AMP, no change is observed for the first 5 minutes, while a significant increase is observed after 10 minutes. Lastly the NAD content is not really affected.

2. *Incubation with noradrenaline 10^{-5} M.*

Total phosphate content decreases abruptly after 5 minutes incubation then remains constant (table III). Inorganic phosphate increases slightly after 5 minutes incubation then remains constant. Labile phosphate content increases only after 10 minutes incubation, while residual phosphate content is not really affected.

ATP content (table IV), contrary to what occurs in the presence of adrenaline remains constant during the first 5 minutes of incubation with noradrenaline then decreases slightly after 5 minutes to remain nearly constant afterwards. ATP content decreases but noradrenaline is not so effective as adrenaline. AMP content decreases after an increase in the first 5 minutes incubation. NAD content decreases between 5 and 10 mi-

TABLE II.

Influence of adrenaline 10^{-5} M on adenylic nucleotides content of rat myocardium.

Incubation time	A. T. P.				A D. P.			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	18	10	10	10	18	10	10	10
$\bar{X} \pm \sigma$	294	247	178	140	151	122	102	87
F	± 10	± 6	± 6	± 6	± 4	± 3	± 4	± 2
		10,13	67,26	22,76		24,40	18,73	10,19
F (0,05)		4,22	4,41	4,41		4,22	4,41	4,41
F (0,01)		7,72	8,28	8,28		7,72	8,28	8,28
Significance		++	+++	++		++	++	+

Incubation time	A.M.P.				N.A. D.			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	18	10	10	10	18	10	10	10
$\bar{X} \pm \sigma$	79	89	103	115	41	42	35	35
	± 3	± 5	± 3	± 5	± 2	± 3	± 1	± 2
F		4,11	4,09	0,48		0,12	5,32	0
F (0,05)		4,22	4,41	4,41		4,22	4,41	4,41
F (0,01)		7,72	8,28	8,28		7,72	8,28	8,28
Significance		—	—	—		—	+	—

A.M.P. between 0 and 10 min : F = 40,2 ; F(0,05) = 4,22 ; F(0,01) = 7,72 ; S = ++.

Results are expressed in µmoles nucleotides/100 g fresh tissue.

nutes incubation, then increases to reach a higher value than the initial value.

In table V is given the evolution of ATP/ADP ratios in the same conditions. It can be seen that this ratio is not really modified after 5 minutes incubation in the presence of either of the two hormones, while between 5 and 10 minutes incubation, there is a net decrease of the ratio, especially with adrenaline.

The free nucleotides content of a tissue is related to the metabolic activity of this tissue and it has even been possible to relate high-energy nucleotides content and cardiac pressure [16]. The influence of catecholamines on the level of these nucleotides seemed, consequently, quite interesting.

In normal myocardium, the ATP, ADP and AMP contents obtained are in agreement with other

TABLE III.

Influence of noradrenaline 10^{-5} M on phosphorylated fractions content of rat myocardium.

Incubation time	Total phosphate				Inorganic phosphate			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	10	8	10	10	10	8	10	10
$\overline{X} \pm \sigma$	863 ± 21	542 ± 7	491 ± 20	538 ± 26	142 ± 13	217 ± 16	240 ± 14	247 ± 15
F		170,64	1,39	0,64		13,79	1,39	0,03
F (0,05)		4,49	4,49	4,41		4,49	4,49	4,41
F (0,01)		8,53	8,53	8,28		8,53	8,53	8,28
Significance		+++	—	—		++	—	—

Incubation time	Labile phosphate				Residual phosphate			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	10	8	10	10	10	8	10	10
$\overline{X} \pm \sigma$	19 ± 2	19 ± 3	32 ± 3	44 ± 4	242 ± 30	260 ± 11	266 ± 11	213 ± 15
F		0,1	7	5,5		0,26	0,18	8,12
F (0,05)		4,49	4,49	4,41		0,49	4,49	4,41
F (0,01)		8,53	8,53	8,28		8,53	8,53	8,28
Significance		—	+	+		—	—	+

Labile phosphate between 0 and 20 min : F = 31 ; F(0,05) = 4,41 ; F(0,01) = 8,28 ; S = ++.

Results are expressed in µg P_i/100 g fresh tissue.

DISCUSSION.

The use of myocardium slices incubated with hormones instead of hormone injection to the whole animal is preferable, in our view, since it prevents, as much as possible, the biochemical consequences due to myocardium anoxia. Most authors, except Pool *et al.* [10], found that the absence of oxygen induces a sharp decrease in ATP content and an increase in inorganic phosphate content [11, 12, 13, 14, 15].

results [17, 18]. The two hormones give an important decrease in the energy-rich phosphorylated compounds, accompanied by a rapid increase in inorganic phosphate content. This is especially true for ATP and to a lesser extent for ADP. NAD and carbohydrate phosphate-ester contents are poorly affected, especially with noradrenaline.

Similar results with adrenaline have been obtained with rat diaphragm uterus [9, 19] and rabbit aorta [20]. The fact that the ATP/ADP

ratio is lowered between 5 and 10 minutes incubation and that the content of total free adenylic nucleotides decreases significantly (35 p. cent after 20 minutes with adrenalin) may be related to the decrease of aerobic metabolism observed in the same experimental conditions [21].

Comparison of adrenaline and noradrenaline effects show that, on the whole, the effects are similar, although noradrenaline is not so effective, especially concerning the P_i accumulation. The decrease in total phosphate, however, occurs more rapidly with noradrenaline, even if, after 20 minutes incubation, the effects of the two hormones are very similar.

These results and the comparison with others favour the view that adrenaline acts in the same manner, whatever the tissue (i.e. diaphragm, uterus, aorta, myocardium), at least as far as phosphorylated intermediates are concerned. This disturbance of the distribution of essential compounds for energy metabolism is similarly observed after induction of experimental atherosclerosis [22] and this may be in relation with the genesis of this disease.

The observed decrease in adenylic nucleotides content of myocardium tissue incubated with catecholamines could be compensated by an increase in the turn-over of these compounds ; this is the subject of the next paper.

TABLE IV.

Influence of noradrenaline 10^{-5} M on adenylic nucleotides content of rat myocardium.

Incubation time	A. T. P.				A. D. P.			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	18	7	14	8	18	7	14	8
$\bar{X} \pm \sigma$	294 ± 10	279 ± 8	221 ± 10	214 ± 10	151 ± 4	136 ± 3	122 ± 4	121 ± 5
F		0,75	15,12	0,24		7,83	6,51	0,006
F (0,05)		4,28	4,38	4,35		4,28	4,38	4,35
F (0,01)		7,88	8,18	8,10		7,88	8,18	8,10
Significance		−	+	−		+	+	−

Incubation time	A. M. P.				N. A. D.			
	0	5 min	10 min	20 min	0	5 min	10 min	20 min
Number of experiments	18	7	14	8	18	7	14	8
$\bar{X} \pm \sigma$	79 ± 3	91 ± 8	91 ± 4	79 ± 5	41 ± 2	41 ± 2	34 ± 2	77 ± 3
F		4,71	0	4,46		0	7,63	23
F (0,05)		4,28	4,38	4,35		4,28	4,38	4,45
F (0,01)		7,88	8,18	8,10		7,88	8,18	8,10
Significance		+	−	+		−	+	++

Results are expressed in µmoles nucleotides/100 g fresh tissue.

TABLE V.

Influence of catecholamines 10^{-5} M on ATP/ADP ratio values measured on rat myocardium after different times of incubation.

Incubation time	0	5 min	10 min	20 min
Adrenaline 10^{-5}M	1,94	2,02	1,74	1,72
Noradrenaline 10^{-5}M	1,94	2,05	1,81	1,76

RÉSUMÉ.

1. Nous avons étudié l'influence des catécholamines (adrénaline et noradrénaline) sur le métabolisme énergétique du myocarde de rat en incubant des tranches fines de ce tissu en présence de ces hormones et en mesurant l'évolution du taux des différentes fractions phosphorylées et des nucléotides adényliques.

2. Nous avons obtenu des effets similaires avec les deux hormones, plus nets, cependant, avec l'adrénaline.

3. La concentration en phosphate total diminue significativement, en présence de catécholamines, cependant que s'accroît le taux du P_i, durant les dix premières minutes d'incubation. Les taux de phosphate labile et phosphate résiduel augmentent au début de l'incubation pour revenir, ensuite, aux valeurs initiales.

4. Les deux hormones provoquent une diminution significative des concentrations en ATP et ADP ; cependant l'effet de la noradrénaline sur le taux d'ATP nécessite un temps d'incubation plus long. Le rapport ATP/ADP diminue après 5 minutes d'incubation et la quantité totale de nucléotides adényliques diminue (35 p. cent après 20 mn d'incubation en présence d'adrénaline).

5. Des résultats semblables ont été rapportés avec d'autres tissus ; ces résultats peuvent expliquer la diminution du métabolisme aérobie que nous avons observée dans les mêmes conditions.

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