INFLUENCE OF ADRENALINE AND HYPOPHYSEAL GROWTH HORMONE ON THE ACID-SOLUBLE PHOSPHATE FRACTIONS OF THE RAT UTERUS*

DANIÈLE GAUTHERON, HUBERT CLAUSER AND PIERRE VOLFIN

Laboratoire de Chimie biologique de la Faculté des Sciences, Paris (France)

In a recent paper[1] the inhibiting action of adrenaline and hypophyseal growth hormone on the endogeneous oxygen uptake of the rat uterus was studied. A similar action of both these hormones has recently been observed on the anaerobic glycolysis of the same organ[2]. Addition of 2,4-dinitrophenol at an uncoupling concentration (10^{-4} *M*) to the incubation medium abolished both these inhibitions. This fact strongly suggested a link between these inhibitions and oxidative phosphorylation phenomena. Hence a possible accumulation of high-energy phosphates and a subsequent decrease of "phosphate acceptors" in this muscle following adrenaline or growth hormone injections had to be considered. The present work** shows that, whereas an increase in high-energy phosphates actually occurs under the influence of growth hormone, adrenaline, quite unexpectedly, promotes a strong rise in inorganic phosphate and a decrease in high-energy phosphates.

EXPERIMENTAL METHODS

Wistar rats of the same strain as before[1] have been used and treated as previously: *l*-adrenaline (91.5 μg per 100 g live weight) was injected intraperitoneally; 45–50 minutes after the injection, the rats were either killed by decapitation or deeply anesthetized by an intraperitoneal injection of veterinary Nembutal*** (3–4 mg per 100 g live weight). Growth hormone was injected subcutaneously (50 μg per animal, dissolved in 0.5 ml of isotonic NaCl at pH 8.0–8.5) 20–24 hours before killing the animal by decapitation. In all cases the uterus was quickly removed immediately after decapitation, or as soon as deep anesthesia was reached, and kept in liquid nitrogen until grinding. In the first case the organ is dipped in the liquid nitrogen about 1 minute after decapitation, whereas the time necessary for the removal of an anesthetized animal's uterus is about 30 seconds.

The uterus is weighed in a deeply frozen state and homogenized with a known volume of 5% trichloroacetic acid (1–1.25 ml per 100 mg fresh weight) in a cooled glass homogenizer, care being taken that the temperature never rises above 0° C. The homogenate is immediately centrifuged at 0° and the clear supernatant ("TCA extract") is suitably diluted for the estimation of the various phosphorus fractions.

The "true" inorganic phosphate was estimated according to LOWRY AND LOPEZ[3]: one volume of "TCA extract" was diluted immediately after centrifugation with 4 volumes of cold 0.1 *M* sodium acetate, the resulting pH being about 4. The usual procedure was then performed immediately, the colour development followed over 10 and 20 minutes and the "true" inorganic phosphate value was extrapolated to 0 time. Blanks and standard phosphorus estimations were run routinely under exactly the same conditions as the real assays (*i.e.* using the same mixture: 1 vol. TCA 5% + 4 vol. sodium acetate 0.1 *M* at pH 4). Moreover, internal standards were run

* Supported by a grant from the Rockefeller Foundation.

** A preliminary note appeared in *Compt. rend.*, 243 (1956) 605.

*** Abbott Lab., London.

References p. 136.

TABLE

Group	*Conditions (number of rats)*	*Number of experiments*	*Average weight (g)*	*Average uterus fresh weight (mg)*	*"True" inorganic phosphorus*
1	Uninjected animals (22)	7	85	87.7	18.1 ± 1.2
2	Adrenaline-injected animals (20)*	6	80	67.1	28.3 ± 1.3 ($P \ll 0.001$)
3	Growth hormone-injected animals (31)**	7	83	86.6	16.9 ± 0.8 ($P > 0.3$)
4	Anesthetized untreated animals (18)	6	89	103.8	15.6 ± 1.2
5	Anesthetized adrenaline-injected animals (12)***	4	81	83.9	27.0 ± 2.1 ($P = 0.001$)
6	Untreated adult animals (7)	6	206	291.4	18.6 ± 1.0
7	Adrenaline-injected adult animals (4)	4	214	422	18.8 ± 0.9

Phosphate is expressed as μg of phosphorus per 100 mg fresh weight of uterus ± standard error of the mean.

P calculated according to Student's formula.

in all assays in order to check the absence of a colour inhibitor and these proved to be in excellent agreement with the actual assays.

"0 min phosphate" ("true" inorganic phosphate + easily hydrolysable esters of the phosphocreatine type) and "7 min phosphate" (energy-rich phosphates of the ATP type, which are hydrolysed by a 7 minutes treatment with N HCl at 100° in a sealed tube) were simultaneously determined using the ZELLER[4] and the FISKE AND SUBBAROW[5] procedures: one volume of the "TCA extract" is diluted with 4 volumes of 10% TCA, as it was established that too high a concentration of Na acetate disturbed the ZELLER method. The difference between the "0 min phosphate" and the "true" inorganic phosphate represents the phosphocreatine-like esters.

RESULTS

Adrenaline-injected rats

Table I shows the influence of an adrenaline injection on the acid-soluble phosphate fractions of the rat uterus as compared with the same fractions in a normal animal. The rats are either killed by decapitation (Groups 1 and 2 or 6 and 7) or anesthetized (Groups 4 and 5).

In young mature rats (average weight < 100 g) there is a striking increase of "true" inorganic phosphate (about 60–70%) under the influence of adrenaline. This increase is highly significant. At the same time the sum of the high-energy fractions (phosphocreatine-like esters and 7 min phosphate) decreases to an extent of 40–50%. This decrease is significant ($P < 0.01$ for the sum of the high-energy phosphates). There seems to be no essential difference between decapitated and anesthetized animals under our experimental conditions.

References p. 136.

I

Zeller *method*			Fiske and Subbarow *method*		
0 min phosphate	*Phosphocreatine-like esters*	*7 min phosphate*	*0 min phosphate*	*Phosphocreatine-like esters*	*7 min phosphate*
23.2 ± 1.0	5.2 ± 1.1	8.2 ± 1.0	23.4 ± 0.9	5.4 ± 1.0	8.7 ± 0.8
32.8 ± 1.5 ($P < 0.001$)	4.0 ± 1.6 ($P > 0.7$)	3.7 ± 1.1 ($P = 0.01$)	32.0 ± 1.4 ($P < 0.001$)	3 ± 1.1 ($P \sim 0.1$)	5.1 ± 1.2 ($P < 0.05$)
24.3 ± 1.2	7.4 ± 1.5 ($P \sim 0.25$)	13.4 ± 2.5 ($P \sim 0.05$)	24.3 ± 1.0	7.4 ± 1.0 ($P \sim 0.2$)	13.4 ± 1.7 ($P \leqslant 0.02$)
21.4 ± 1.1	5.8 ± 0.8	6.5 ± 1.6	21.6 ± 0.9	6.7 ± 1.0	6.5 ± 1.3
28.3 ± 0.8 ($P = 0.001$)	1.3 ± 1.7 ($P < 0.05$)	5.6 ± 1.0 ($P < 0.7$)	28.5 ± 1.5 ($P = 0.001$)	1.5 ± 0.6 ($P < 0.01$)	3.9 ± 0.5 ($P \sim 0.2$)
22.6 ± 0.7	3.6 ± 1.2	7.8 ± 1.7	21.3 ± 0.5	2.6 ± 0.6	7.9 ± 0.8
23.1 ± 1.5	4.3 ± 1.5	8.2 ± 1.2	22.3 ± 1.0	3.5 ± 1.1	6.0 ± 2.2

* P values for groups 1 and 2.
** P values for groups 1 and 3.
*** P values for groups 4 and 5.

In adult animals (average weight > 200 g; Groups 6 and 7) adrenaline has no influence whatsoever on the phosphate fractions of the uterus. This result agrees with the previously reported fact[1] that adrenaline has no influence on the endogeneous oxygen uptake of the uterus of the same kind of rats.

Growth hormone injections

Table I (Groups 1 and 3) shows the influence of growth hormone injections on the uterus of young mature rats. There is no change in inorganic phosphate. The phosphocreatine-like esters show a tendency to increase (+ 40%), although this increase is not statistically significant, owing to the small quantities of these esters. The 7 min phosphate shows a definite (+ 60%) and significant increase, as compared with the untreated animals.

DISCUSSION

The striking differences between the action of adrenaline and growth hormone on the phosphate fractions of the uterus definitely rule out the possibility of adrenaline acting through an increased secretion of hypophyseal growth hormone. This fact agrees with the observation that it has never been possible to obtain the inhibiting action of growth hormone within as short a period after the injection as with adrenaline[1]. It does not rule out, of course, a possible permissive action of the hypophysis on the adrenaline effect.

References p. 136.

The inhibiting action of adrenaline on the uterine metabolism and the release of the inhibition by uncoupling concentration of 2,4-DNP[1] had been tentatively explained by an inhibiting action of adrenaline on the oxidative phosphorylations, possibly involving an accumulation of high-energy phosphates. The present results show that there is no such accumulation. On the contrary, the high-energy phosphates are significantly decreased after adrenaline injections, and there is a striking increase of inorganic phosphate. Nor can the well-known effect of adrenaline on muscle phosphorylase[6], which has been recently extended to the uterus[7], offer any ready explanation for this inorganic phosphate accumulation, for it has been shown[8] that this action should rather result in a decrease of inorganic phosphorus.

Thus, if the inhibition of metabolism by adrenaline is linked to oxidative phosphorylation phenomena, as the action of 2,4-DNP suggests, it must be at a step previous to the phosphorylation of ADP to ATP. But there might also be a decrease in phosphate acceptors owing to the conversion of ADP to ATP plus adenylic acid, resulting from myokinase activity. The present state of the work does not enable us to give a definite answer to this question.

In the case of growth hormone injections, there is a significant increase of high-energy phosphates in the uterus. This may account for the inhibition of metabolism (oxygen uptake and glycolysis) and agrees with the 2,4-DNP effect on the oxygen uptake of inhibited organs. It definitely suggests that the slowing down of metabolism may be due to the lack of phosphate acceptors, like ADP. However, the definite proof of this hypothesis requires further investigations.

N.B. Since this paper was sent to the editor, further experiments proved, quite surprisingly, that an uterus kept at 0° shows a higher energy-rich phosphate content than a frozen one. However, this fact does not affect the hormonal actions reported in this paper.

ACKNOWLEDGEMENT

The authors are indebted to Professor C. H. Li for his generous gift of pure hypophyseal growth hormone.

SUMMARY

Adrenaline injections are followed by an increase of "true" inorganic phosphate and a decrease of high-energy phosphate fractions in the rat uterus. Growth hormone injections provoke an increase of high-energy phosphates in the same organ. The relationship between these changes and oxidative phosphorylation phenomena is discussed with regard to the growth hormone- and adrenaline-promoted inhibitions of the uterine metabolism, which have been previously reported.

REFERENCES

[1] D. GAUTHERON AND H. CLAUSER, *Biochim. Biophys. Acta*, 22 (1956) 123.
[2] D. GAUTHERON AND H. CLAUSER, unpublished data.
[3] O. H. LOWRY AND J. A. LOPEZ, *J. Biol. Chem.*, 162 (1946) 421.
[4] E. A. ZELLER, *Helv. Chim. Acta*, 32 (1949) 2512.
[5] C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
[6] E. W. SUTHERLAND, *Ann. N.Y. Acad. Sci.*, 54 (1951) 693.
[7] J. L. KOSTYO AND S. L. LEONARD, *Endocrinology*, 56 (1955) 616.
[8] G. T. CORI AND B. ILLINGWORTH, *Biochim. Biophys. Acta*, 21 (1956) 105.

Received September 27th, 1956