ENZYMOLOGICAL STUDIES ON THE CONTRACTION OF THE PREGNANT UTERINE MUSCULATURE

—ON THE INFLUENCES OF ANORGANIC SUBSTANCES UPON ACTOMYSIN-ADENOSIN-TRIPHOSPHATASE (AM-ATPase)—

BY

Kyushiro FUJII*, Koichi KATO* and Koichi HIRAKAWA*

In the previous report the authors prepared a kind of raw AM-ATPase out of uterine muscle of the rat during pregnancy and parturition and confirmed a remarkable increase of AM-ATPase activity in the latter[1]. The present paper describes the influences of various anorganic substances on the activity of the raw enzyme prepared from pregnant uterus of the rat.

EXPERIMENTAL METHOD AND MATERIALS

Applied substrate, buffer and raw enzyme solution were the same to those described in the previous report[1].

The composition of experimental solution was as follows,

enzyme soluton	1.0 ml
tris-buffer	4.0 ml
6/10 M KCl	1.0 ml
added substance	1.0 ml
distilled water	1.0 ml

After keeping at 37 C for one hour 1.0 ml of M/400 ATP was added to the solution and it was incubated for 15 minutes. Then, it was deproteinized by mixing with the same volume of 10% trichloro-acetic acid. The liberated anorganic phosphoric acid in the solution was colorimetrically determined according to Youngburg & Youngburg method[2].

EXPERIMENTAL RESULTS

The influences of the following substances on the raw AM-ATPase were investigated, and the result was as follows.

Influences of Ca^{++} and Mg^{++}: The changes of AM-ATPase activity were examined under various concentration of $CaCl_2$ or $MgCl_2$. The results

* 藤井久四郎, 加藤宏一, 平川浩一: Dept. of Obst. & Gynec. (Chief: Prof. K. Fujii) School of Medicine.

Received for publication, Dec. 15 th 1960.

are shown in Fig. 1, 2 and 3. In the experimental solution three kinds of concentration of KCl as 0.12, 0.3 and 0.6 M were applied. Ca^{++} and Mg^{++} brought about maximal activation of AM-ATPase at terminal concentration of M/1000 or M/10000.

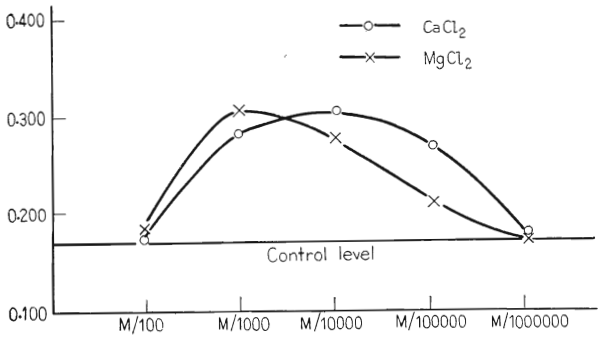

Fig. 1. Influence of $CaCl_2$ and $MgCl_2$ in solutlon including 0.12 M. KCl

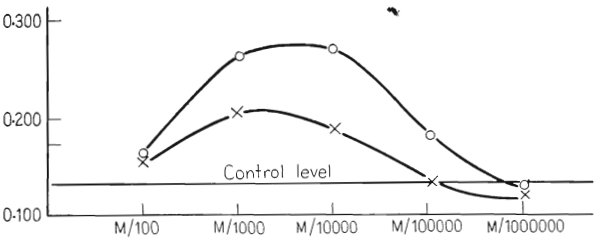

Fig. 2. Influence of CaCl and MgCl in solution including 0.3 M. KCl

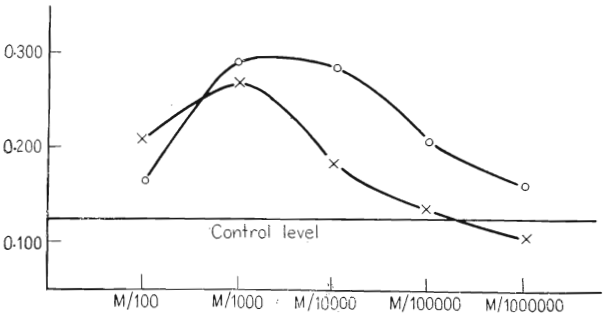

Fig. 3. Influence of $CaCl_2$ and $MgCl_2$ in solution including 0.6 M. KCl

Influence of $Mn.^{++}$: MnCl was added to experimental solution. The result is tabulated in Table 1. Mn^{++} activated activity of AM-ATPase at terminal concentration of M/10000, but inhibited it at M/100.

Influence of $FeCl_3$ *and* $Fe_2(SO_4)_3$: The results are shown in Table 2.

Table 1. Influence of MnCl on AM-ATPase activity

Terminal concentration of $MnCl_2$	ε
M/100	0.138
M/1000	0.291
M/10000	0.311
M/100000	0.275
control	0.234

Table 2. Influence of $FeCl_3$ and $Fe_2(SO_4)_3$

Terminal concentration of added substance	ε	
	$FeCl_3$	$Fe_2(SO_4)_3$
M/1000	0.097	0.103
M/10000	0.170	0.164
M/100000	0.180	0.181
control	0.208	0.208

Under these conditions Fe^{++} and Fe^{+++} inhibited always the activity of the enzyme.

Influence of $CuSO_4$, NaCN and NaF: In Table 3 the results are tabulated. $CuSO_4$ inhibited the activity of AM-ATPase, while NaCN could activate it. On the other hand NaF inhibited it in a slight degree at terminal concentration of M/100 and had no influence at M/1000.

Table 3. Influence of $CuSO_4$, NaCN and NaF.

Terminal concentration	ε		
	$CuSO_4$	NaCN	NaF
M/100			0.176
M/1000	0.007	0.320	0.197
control	0.180	0.180	0.210

Influence of NH_4OH and KI: As shown in Table 4 no influence of these substances was confirmed on the enzyme activity.

Table 4. Influence of NH_4OH and KI

Terminal concentaation of added substauce	ε	
	NH_4OH	KI
M/100	0.202	
M/1000	0.212	0.208
M/10000	0.210	
control	0.210	0.210

Influence of dialysis on activity of the raw enzyme: The raw enzyme was subjected to dialysis against running water for 17 hours, but there was no change of activity between before and after dialysis as seen in Table 5.

Influence of acetone treatment on activity of the raw enzyme: The raw

Table 6. Activity of AM-ATP ase actone powder

Added substance	ε
M/1000 $MgCl_2$	0.004
M/1000 $CaCl_2$	0.002
distilled water	0.000

Table 5. Influence of dialysis

Raw enzyme	ε
Before dialysis	0.206
After dialysis	0.201

preparation of AM-ATPase was treated with ice-cold acetone and desiccated to get acetone powder of the enzyme, activity of which was examined adding $MgCl_2$ or $CaCl_2$ in terminal concentration of M/1000. As shown in Table 6 the activity of AM-ATPase was almost completely inhibited if desiccated by acetone treatment.

Discussion

We[1] confirmed that activity of AM-ATPase prepared from pregnant uterine muscular layer of the rat was significantly elevated at physiological and mechanically induced contraction. In view of spontaneous onset of labor it seems to be interesting to examine what factors have to do with the enzyme activity of the pregnant uterus.

According to many investigations heretofore Ca^{++} or Mg^{++} are said to accelerate AM-ATPase activity of skelet muscles. In the present experiment the raw enzyme of the pregnant uterine muscle was activated or inhibited according to the concentration of Ca^{++} or Mg^{++}. As reported already, if atomyosin of the skelet muscle can be activated by Ca^{++} and Mg^{++}, but myosin only by Ca^{++} out of two ions, the enzyme concerned in the pregnant uterus seemed to exist as a unit of actomyosin when extracted with regards to its attitude for the both ions.

Mn^{++} activated the enzyme at proper concentration of 10^{-4} M and inhibited it at 10^{-2} M. NaCN exerted an activating influence on the enzyme. However, the action of NaCN seemed to be indirect and to remove inhibiting factors, making a kelating compound with inhibiting substance, as it is the case with alkaline phospho-monoesterase. NaF, Fe^{++} and Cu^{++} showed more or less inhibiting influences on the enzyme, while KI and NH_4OH were inactive at terminal concentration of 10^{-3}. Of course, such influences have to be investigated with a purified enzyme substance further. However, these results may offer som important facts possibly for understanding the role of AM-ATPase in the uterine contraction.

An attempt to isolate a co-enzyme by dialysing the raw enzyme con-

cerned did not succeed. The co-enzyme, if existed, might be difficult to isolate in such a way. Another attempt to isolate co- and apo-enzyme was in vain, because the raw enzyme got inactive after treated with acetone. But it must be studied further.

Summary

1. Raw enzyme of acto-myosin ATPase extracted out of the pregnant uterine muscle of the rat was investigated in the attitude for anorganic ions.
2. Its activity was accelerated by Mg^{++}, Ca^{++}, Mn^{++} and NaCN.
3. Its activity was inhibited by Fe^{++} and Cu^{++}.
4. KI and NH_4OH had no influence.
5. Dialysis could not isolate Co-enzyme out of the enzyme.
6. Acetone powder of the raw enzyme was inactive.

References

1) Fujii, K., Kato, K. and Hirakawa, K.: Bull. Tokyo Med. & Dent. Univ. 8: (1) 49, 1961.
2) Youngburg and Yungburg, J. Lab. Clin. Med., 16, 158 (1930).
3) Nagai, T.: Physico-chemistry, Igakushoin, 1956.
4) Akamatsu, S., Aso, Y.; J. Biochem., 43: 1, 1956.
5) Akamatsu, S., Murakoshi, Y.: Arch. Biochem. & Biophysics, 69: 353, 1957.