ENZYMOLOGICAL STUDIES ON THE CONTRACTION OF THE PREGNANT UTERINE MUSCULATURE

—ON THE INFLUENCE OF PLACENTAL EXTRACT ON ACTOMYOSIN-ATPase—

BY

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With purpose of investigating constraction of the pregnant uterus from enzymological stand point the authors[1,2] confirmed that the activity of actomyosin ATPase prepared from rat's pregnant uterus was significantly elevated during contraction, and that the enzyme was activated by Mg and Ca ions and inhibited by Fe and Cu ions. In the present experiment the same enzyme activity was observed under influences of anorganic ions derivd from the placental tissue.

EXPERIMENTAL METHOD AND MATERIAL

Substrate: Adenosine triphosphate sodium salt (Wako pure chemical) was used.

Buffer: Tris (hydroxymethyl) aminomethane-HCl buffer (Ph 7.0) was used.

Enzyme solution was prepared as described in the previous paper[1].

Composition of experimental solution was as follows,

enzyme solution	1.0 ml
tris-buffer	4.0 ml
6 M/10 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 into the solution, which was incubated for 15 minutes.

Determination: The solution above was deproteinized by mixing with the same volume of 10% trichloro-acetic acid. Then, liberated anorganic phosphoric acid was colorimetrically determined by Youngburg & Youngburg method[3]. Homogenate of the placenta at the end of pregnancy of the rat or woman was centrifuged for 30 minutes at 12000 r.p.m. The supernatant solution or dialysed external solution was used in the following experiments.

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Experimental Results

In order to remove ATPase included in the placental homogenate of the rat it was heated, and the temperature at which ATPase activity got completely lost was determined (Table 1). The activity of the enzyme in the placenta was completely inhibited by heating at 70°C for 5 minutes or at 60°C for 10 minutes.

Table 1. Influence of heating on ATP ase activity in the supernatant of placental homogenate of the rat.

Temperature C.	Heating 5 minutes	Heating 10 minutes
room temp.	*0.168	
40°C	0.174	
50°C	0.098	
60°C	0.041	*0.000
70°C	0.000	0.001

* extinction coefficient

The enzymic inactive supernatant prepared by heating as above was added to the enzyme solution of AM-ATPase of the rat. As seen in Table 2, ATPase activity of the placenta was quite inhibited. This experiment showed that the placenta at the end of pregnancy of the rat had some substances to be able to inhibit the AM-ATPase.

Table 2. Influence of heated homogenate of placenta (60°C 10 minutes) on AM-ATP ase.

	Extinct. coeff.
control AM-ATP ase solution	0.165
heated supernatant solution added	0.089

The homogenate of placenta at the end of pregnancy of the rat was subjected to autolysis by keeping in incubator at 37°C for 48 hours, supernatant of which was compared with that further heated at 60°C for 10 minutes in the enzyme activity of ATPase (Table 3). The autolysis under the above condition inhibited the enzyme activity.

Table 3. Influence of autolysis on AM-ATP ase

Solution	Extinct. coeff.
control solution of AM-ATP	0.165
autolysed homogenate added	0.000
autolysed, heated homogenate added	0.062

The rat placenta (3 g) was cleared of blood as complete as possible and homogenated. The homogenate was subjected to dialysis against distilled water in refrigerator for three days, the external solution of which was concentrated by heating to 10 ml. Its 1.0 ml was added to experimental

solution and the influence on actomyosin ATPase was examined (Table 4). From the result it was confirmed that there was in the dialysed external solution of placental homogenate at the end of pregnancy of the rat no substance which could influence on the activity of actomyosin ATPase of its pregnant uterus.

Table 4. Influence of dialysed external solution of rat's placenta on actomyosin AM-ATPase of rat's pregnant uterus.

Group	Control	Dialysed solution added
I	0.143	0.140
II	0.210	0.210
III	0.165	0.172

Using the placenta of the rat found just during labor similar experiment was carried out. During labor the placenta seemed to have some substances which could activate actomyosin ATPase of pregnant uterus of the rat (Table 5).

Table 5. Influence of dialysed external solution of rat's placenta during labor on actomyosin ATPase

control	0.178
added	0.324

As to full-term delivered placenta of the woman, inhibiting effect of heating on ATPase of the placenta itself was examined under the similar procedure to the rat's placenta. Heating at 60°C for 10 minutes inhibited ATPase activity of the human placenta (Table 6).

Table 6. Effect of heating on the supernatant of homogenate of full-term delivered human placenta

Temperature C	Heating for 5 minutes	Heating for 10 minutes
room temperature	0.133	
40°C	0.138	
50°C	0.106	
60°C	0.060	0.006
70°C	0.050	0.000

The supernatant fluid of homogenate of human full-term placenta heated at 60°C for 10 minutes could inhibit AM-ATPase activity of the rat's pregnant uterus as shown in Table 7.

The homogenate and slices of full-term delivered placenta cleared of blood were subjected to dialysis against distilled water in refregerator for two days, and their effect on the actomyosin ATPase of rat's pregnant

Table 7. Influence of heated homogenate of human placenta (60°C, 10 minutes) on ATPase of the pregnant uterus of the rat

Placental homogenate	Extinction coeff.
control solution of AM-ATPase	0.242
the same mixed with heated supernatant	0.142

uterus was investigated (Table 8). The result showed that the dialysed external fluid of homogenates or slices of delivered full-term placenta could activate AM-ATPase of the pregnant uterus of the rat.

Table 8. Influence of dialysed external fluid of human placenta on AM-ATPase of rat's pregnant uterus

Control	Homogenate	Slice
0.152	0.240	0.284
0.086		0.125
0.158		0.200
0.109		0.157
0.099		0.225

In cesarean sections carried out at the end of pregnancy preceding onset of labor the placenta was taken out and in a way as described above dialysed as slices for 3 days. The dialysed external fluid was examined in its effect on AM-ATPase of pregnant uterus of the rat (Table 9). The dialysed external fluid of human placenta could not activate the rat's AM-ATPase before onset of spontaneous labor.

Table 9. Influence of dialysed external fluid of human placenta before onset of labor on AM-ATPase of rat's pregnant uterus

Case	Control	Added
I	0.150	0.143
II	0.174	0.165
III	0.213	0.208

The same placenta removed at cesarean section before onset of labor was kept in incubator at 37°C for 17 hours, and then dialysed as in the above experiment. As shown in Table 10, the dialysed external fluid

Table 10.

Control	Dialysed immediately	Dialysed after keeping at 37C
0.150	0.143	0.108
0.260	0.255	0.195

seemed to inhibit some-what the AM-ATPase activity of rat's pregnant uterus.

Dialysed internal and external fluid of spontaneously delivered human

placenta of full-term were compared together in their effects on AM-ATPase of rat's pregnant uterus (Table 11). The external fluid activated and the internal fluid inhibited AM-ATPase of rat's pregnant uterus.

Table 11.

Control	External fluid	Internal fluid
0.120	0.186	0.050

Discussion

As it is the case with enzymes in general, the actomyosin-ATPase activity of the pregnant uterus was confirmed to be highly influenced by anorganic ions, namely accelerated by Ca^{++}, Mg^{++} and Mn^{++} while inhibited by Fe^{++} and Cu^{++} according to our previous experiments[2], too. If it is assumed that some shiftings of balance among such ions have to do with the activity of AM-ATPase of the pregnant uterus, hence the onset of labor, the placenta seems probably to be the site of regulating the mechanism. That is why the placenta was investigated in relation to its influence on the enzyme in the present experiment.

In order to investigate the influence of placenta on AM-ATPase, the same enzyme possibly included in itself had to be excluded, and it was realized by heating the placental material under a condition. The placenta of the rat preceding to onset of labor had some substances, which could inhibit the AM-ATPase of the rat's pregnant uterus.

When homogenate of rat's placenta before onset of labor was subjected to dialysis, the external fluid exerted neither accelerating nor inhibiting effect on AM-ATPase of rat's pregnant uterus. It is interesting that the dialysed external fluid which includes accelerating and inhibiting ions such as Ca^{++}, Mg^{++}, Fe^{++} and Cu^{++} can manifest no influence on the enzyme concerned. However, it was quite different in case of the experiment with rat's placenta after onset of labor, the dialysed external fluid of which surely accelerated AM-ATPase activity of rat's pregnant uterus.

Such relations were similar when human placenta before or after onset of spontaneous labor was taken into experiment, although here AM-ATPase prepared from human pregnant uterus could not be used.

These results seem to be consistent with our previous report[1] that the activity of AM-ATPase of the pregnant uterus was elevated rapidly with onset of labor in the rat.

The dialysed external and internal fluid of spontaneously delivered human placenta accelerated and inhibited AM-ATPase of rat's pregnant uterus respectively, while the placental homogenate inhibited somewhat the enzyme. The delicate mechanism of accelerating or inhibiting the enzyme

as a whole is quite obscure in our stage of investigation. The difficult problem must be approached step by step further.

Summary

1. The supernatant of homogenate of the placenta at the end of pregnancy of the rat inhibited actomyosin-ATPase activity of the rat's pregnant uterus.
2. The supernatant of autolysed placental substance at the end of pregnancy of the rat inhibited the actomyosin-ATPase activity.
3. The external dialysed fluid of placenta removed preceding to onset of labor of the rat had no effect on AM-ATPase, while that of placenta delivered naturally activated it.
4. The external dialysed fluid of human full-term placenta removed by cesarean section preceding to onset of labor did not influence on AM-ATPase activity. However, it was somewhat inhibited when the placenta was kept, in advance, in incubator at 37°C for 17 hours.
5. Using spontaneously delivered human placenta at term the dialysed external fluid activated AM-ATPase of the rat's pregnant uterus, but the internal fluid inhibited it.

References

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3) Youngburg and Youngburg, J. Lab. Clin. Med., 16, 158 (1930).