ENZYMOLOGICAL STUDIES ON THE CONTRACTION OF THE PREGNANT UTERINE MUSCULATURE

ON THE ACTIVITY OF ACTOMYOSIN-ATPase DURING PREGNANCY AND PARTURITION

BY

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Recently it is reported that in the muscular contraction the contractile protein complex actomyosin (AM) and adenosine-tri-phosphate (ATP) as a source of high energy play important rôle[1,2,3,5]. However, most of these investigations are restricted to the skelet muscle and there are only a few reports on the involuntary muscle[7,8,9,10]. As for the onset of labour or premature interruption of pregnancy there have been offered not a few hypotheses, which cannot, however, explain the complicated mechanism or cause either. In the present paper the difficult problem of the uterine muscular contraction was tried to approach from the activity of actomyosin ATPase in the different phases of gestation of the rat.

EXPERIMENTAL METHOD AND MATERIALS

Substrate: Adenosine triphosphate-sodium salt (prepared by Wako) was used as a substrate.

Buffer: Tris (hydroxymethyl) amino-methane-HCl-buffer was used for buffer solution.

Enzyme solution: According to Szent-Györgyi's method[4] the rat was killed by decapitation. Immediately the pregnant uterus was removed, separating from fetus and placenta. After washing with destilled water the uterine muscle was minced and suspended with Weber-Edsall solution (0.6 M KCl, 0.01 M Na_2CO_3 and 0.04 M $NaHCO_3$) ten times in volume, being homogenized under ice-cold temperature and kept in refregerator for 17 hours. This extract was used for the following experiment as a raw enzyme solution of actomyosin ATPase.

Experimental solution: Its composition was as follows,

enzyme solution	1.0 ml
tris-buffer	4.0 ml

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Received for publication, Dec. 15th 1960.

distilled water	3.0 ml
6/10 M KCl	1.0 ml

This solution was kept at 37°C for 10 minutes and then 1.0 ml of M/400 ATP salt was added, being kept further at 37°C.

Method of derermination: The experimental solution described above was added to the same volume of 10% trichloroacetic acid for deproteinization. The anorganic phosphoric acid liberated from ATP in the solution was determined by the colorimetric method of Youngburg[5].

Experimental results

Optimum pH in the activtiy of AM-ATPase: The raw enzyme, which was prepared from pregnant uterine muscles of the rat, was examined on the optimum acidity for displaying activity. The result is shown in Table 1. The activity of the enzyme was only relatively accelerated under alkaline condition without showing no particular peak.

Table 1.
Optimum acidity for AM-ATPase of pregnant uterus of the rat (ε extinction coefficient)

pH	ε
5.3	0.296
6.0	0.331
6.6	0·338
6.9	0.348
7.4	0.358
8.0	0.378
8.4	0.381
9.0	0.380

Activity of AM-ATPase in different period of gestation: The activity of AM-ATPase of the rat's uterus at the end of pregnancy and during labour was compared in the different animals as shown in Fig. 1. It was elevated about 3 to 4 times during parturition in comparison with that at the end of pregnancy while individual variations were quite minimal. The activity increased in proportion with incubation time at least until 15 minutes.

Change of AM-ATPase actvity in the course of gestation: AM-ATPase activity was observed in the same animal, in which the onset of labour with expulsion of fetus took place accidentally 3 hours after unilateral horn of pregnant uterus was removed for investigation. The bilateral horns of the rat were examined on activity of the enzyme. As shown in Fig. 2 the activity was also elevated about 4 times more during labour than at the end of pregnancy. Although such a transitionary period from pregnancy to

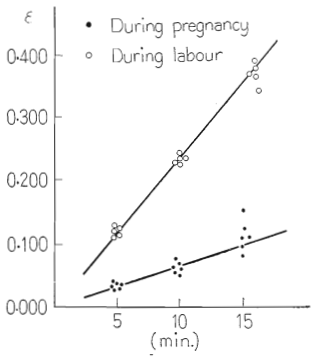

Fig. 1. AM.ATPase during pregnancy parturition

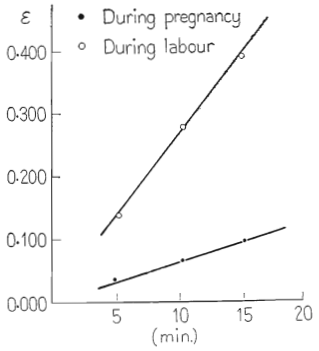

Fig. 2. The AM-ATPase activity of the same rat during pregnancy and labour

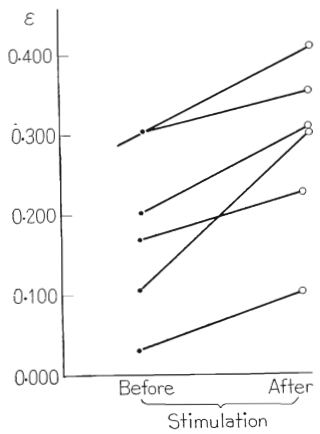

Fig. 3. Change of AM-ATPase activity of pregnant uterus following mechanical stimulation

labour was caught by chance, it is interesting to note that the enzyme activity increased abruptly so much within three hours.

AM-ATPase activity of pregnant uterus following mechanical stimulus: After removing the unilateral uterine horn at the end of pregnancy for preparing enzyme the other horn with fetus in situ was exposed to some mechanical stumuli. When the fetus were pressed out as a result of induced uterine contraction, the uterine muscle of this horn was subjected to enzyme extraction. Both the horns were compared with each other in the activity of AM-ATPase. As seen in Fig. 3, the elevation of AM-ATPase activity of uterine muscle following mechanical stimulation was quite similar to that of normal delivery in quality.

Discussion

Kühne (1868)[1] succeeded in extracting a constituent protein called myosin out of skelet muscle by concentrated salt solution. Engelhardt (1939)[3] found out that actomyosin had ATPase activity. Szent-Györgyi[4,5], Needham[7] and others[2] investigated the physico-chemical characters of actomyosin and confirmed that muscular contraction took place as a result of interaction between AM and ATP. Most of these investigations were performed on the skelet muscle and there have been only a few studies on the uterine muscle (Csapo[7,8,9,10], Izawa[11] and others). AM of skelet and uterine muscle is assumed to be almost similar in their physico-chemical characters, but it must be investigated further. Muscular contraction itself may be caused through the similar energetic mechanism, but underlying and regulating conditions seem to be quite different.

Csapo[7,10] determined AM of uterine muscle in various periods and reported that AM increased in the cause of pregnancy as much as six times towards the end of pregnancy and decreased following delivery in quantity. In the present paper we tried to look into AM-ATPase activity of uterine muscle of the rat in order to catch AM in function. Because it is evident that increase of AM in quantity itself does not mean uterine contraction even at the end of pregnancy, but only shows elevated potentiality of contraction at the time of labour. According to our present investigation with raw AM-ATPase of the rat's uterus the activtiy of the enzyme got abruptly and remarkably elevated with the onset of labour or uterine contraction under either physiological or experimental condition. It seems to be interesting that AM-ATPase activtiy has intimate relations to actual uterine contraction. If various conditions, under which the activity of the enzyme gets abruptly elevated, could be detected, the cause of the onset of labour might be more approached.

Summary

1. AM-ATPase was prepared as raw substance out of the rat's uterus at the end of pregnancy and during parturition, and its activity was compared.

2. The activty of the enzyme got distinctly elevated in the presence of labour as much as four times than prior to the onset of labour. Among several animals there observed merely few variations in this regard.

3. When in the same rat an unilateral horn was removed at the end of pregnancy and another horn after three hours showing by chance parturition in progress, the increase of AM-ATPase in activity was also remarkable in

the latter.

4. When pregnant uterus was exposed to mechanical stimuli resulting in contraction similar to natural labour, elevation of AM-ATPase activity was proved almost in the same degree, too.

This paper was read in the 11th General Congress of Japan Obsterical and Gynecological Society, 1959.

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