THE ACTIN CONTENT OF THE MYOMETRIUM

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The smooth muscle of the uterus, like other forms of smooth muscle, is known to differ considerably in its protein composition from skeletal muscle. The most characteristic feature of smooth tonic muscle is its low content of actomyosin [2, 3, 4, 5, 7, 9] and its relatively high content of water-soluble myofibrillary proteins.

The study of the fractional composition of the proteins of the myometrium has attracted, in recent years, the attention of many research workers [1, 7, 8, 11 and others]. These authors have confirmed the view that the muscle of the nonpregnant uterus has a low content of contractile proteins — myosin and actomyosin. The presence of considerable quantities of tropomyosin in the myometrium has been pointed out on several occasions [12, 14 and others].

The work of Snellman and Tenow differs from that of other workers in that these authors found considerable amounts of free actin (up to 35-40%) besides a protein complex (which they called actotropomyosin) in extracts obtained from the muscle of the uterus with solutions of high ionic strength. According to these workers' findings, action readily passes into solution even during brief extraction of minced muscle with 0.5M KCl. This view, however, is not in accordance with certain facts.

In the present paper we describe findings from which it will be seen that the proteins extracted from Weber's solution from the smooth muscle of the uterus (both pregnant and nonpregnant) do not have the power to react with myosin preparations to form actomyosin: the viscosity and the dissociation power of extracts of smooth muscle of the uterus during reaction with ATP were not appreciably increased by the addition of myosin solutions to them. This makes Snellman and Tenow's view of the presence of any significant amount of free actin in 0.5 M KCl extracts of the smooth muscle of the uterus most unlikely.

In our experiments, in order to define the protein composition of the myometrium, proteins extracted from the uterine muscle with Weber's solution were subjected to fractionation by means of paper electrophoresis. These investigations also gave no grounds for relating the special feactures of the proteins of the myometrium to the high content of "readily extractable" actin in the smooth muscle of the uterus.

EXPERIMENTAL METHOD

The contractile proteins were isolated from the striped muscle and uterine muscle of rabbits (nonpregnant and pregnant). Myosin was obtained by extraction of minced skeletal muscles for 7-8 minutes with three times their volume of Weber's solution in the cold. Actin was prepared by Straub's method. The actin was activated with the salts KCl and $MgCl_2$ (final concentration of KCl — 0.6M, $MgCl_2$ — 0.0005 M). Saline extracts were prepared from the uterine muscle, carefully freed from mucous membrane. Minced muscle tissue was extracted immediately (or after one preliminary washing with 0.3 M KCl solution) with three times its own volume of

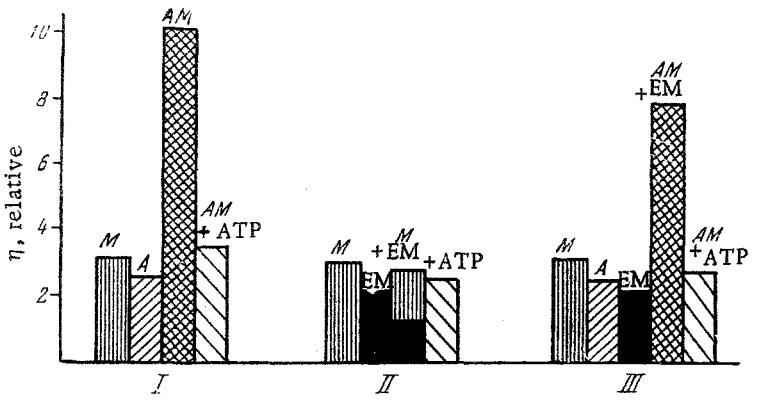

Fig. 1. Changes in the relative viscosity of myosin solution after reaction with actin and with uterine muscle extract. M) Myosin; A) actin; AM) actomyosin; EM) uterine muscle extract.

Weber's solution for 20-22 hours. Myosin and actin solutions from the skeletal muscle, and also saline extracts of uterine muscle, were prepared with closely similar viscosity. The viscosity was measured in an Ostwald viscosimeter at a temperature of +17°C. A 1% solution of ATP was added directly into the viscosimeter. The myosin and actin solutions were added together in proportions of 3:1 by volume (in order to obtain an "artificial" actomyosin), and the uterine muscle extracts and myosin solution were combined in proportions of 1:1.

Electrophoresis of the proteins of the myometrium was carried out under the following conditions. The uterine muscle was cut up finely with scissors, pounded with three times its volume of Weber's solution and extracted in the cold for 24 hours. The suspension was squeezed through 3 layers of gauze and then centrifuged at 4000 rpm. The centrifugate obtained was decanted from the precipitate and dialyzed against a buffer solution for 24 hours. After dialysis, the extract was again centrifuged to ensure complete removal of suspended particles. The protein concentration of the solution was usually 1.2-1.8%. Electrophoresis was carried out in a type LKB 3276 apparatus. The protein solutions, in volumes of 0.03-0.04 ml, were applied to strips of paper measuring 40x 410 mm (Schleicher and Schüll No. 2043 B electrophoresis paper). Electrophoresis was performed in a phosphate buffer at pH = 7.8 and at an ionic strength of 0.35 for 24 hours, with a potential gradient of 4.0-4.5 v/cm and a current of 0.8 ma/cm^2 cross section of paper. The dry electrophoregram was fixed at a temperature of 105° for 15 minutes and then stained in the usual manner with thymol blue. The staining density of the bands corresponding to the different protein fractions was determined by means of a densitometer.

EXPERIMENTAL RESULTS

The results of one of the experiments are shown graphically. As may be seen from Fig. 1(variant 1 of the experiment), myosin solution from skeletal muscles, when mixed with actin solution (in proportions of 3:1) underwent a sharp change in its relative viscosity η as a result of the formation of artificial actomyosin. On the addition of ATP the viscosity of the formed actomyosin fell to the viscosity of the original solutions.

If the myosin solution was mixed with a saline extract of the uterine muscle of a nonpregnant rabbit (according to Snellman and Tenow's findings, this extract contained 35-40% of free actin), in this case no change was observed in the viscosity, even when the myosin solution and the saline extract of the uterine muscle were mixed in proportions of 1:1 (variant II of the experiment). The viscosity assumed an average value, which was determined by the initial viscosity and by the volumes of the solutions mixed. In precisely the same way the viscosity of the solution obtained showed practically no fall after addition of ATP. The insignificant fall in the viscosity which might have been observed after addition of ATP to the solution was due to the presence of a small quantity of actomyosin in the extract of the uterine muscle.

We obtained similar results in experiments with extracts of the uterus of pregnant rabbits.

The supposition that the extracts of the myometrium contained both actin and a hypothetical inhibitor, in the presence of which the reaction of combination of actin with myosin was retarded, was tested by variant III

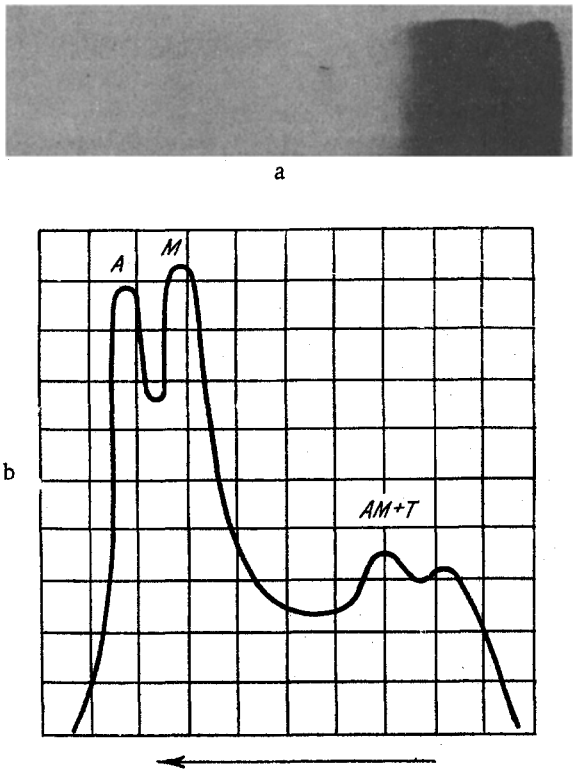

Fig. 2. Separation of the protein fractions of an extract of the uterine muscle of a nonpregnant rabbit (on a strip of paper). a) Stained electrophoregram; b) curve obtained by densitometry of the paper electrophoregram: A) Myoalbumin; M) myogenic fraction ; AM + T) actomyosin + water-soluble myofibrillary proteins.

of the experiment. In this series of experiment myosin solution, actin solution and extract of myometrium were mixed in proportions of 2:1:2. As might have been expected, a highly viscous solution of actomyosin was obtained, which had its relative viscosity sharply reduced by the addition of ATP.

This suggested that Snellman and Tenow's conclusion [12], of the high content of "readily extracted" actin in the myometrium, is incorrect. The distinctive features of the protein composition of the smooth muscle of the uterus were, evidently, in no way connected with the presence of a large amount of actin in muscle of this type.

The results of the electrophoretic investigation of the proteins of the myometrium (Fig. 2), extracted from the uterine muscle by salt solutions of high ionic strength, showed that the proteins of the smooth muscle of the uterus mainly consist of 3 fractions: myoalbumin A, heterogeneous myogen fraction M and actomyosin fraction AM. The last, according to the findings of I. I. Ivanov and his co-workers, consists of a small quantity of actomyosin combined with the water-soluble myofibrillary proteins of the myometrium (fraction T), mainly with tropomyosin, and with a new, water-soluble myofibrillary protein (Δ-protein, X-protein), whose properties have recently been studied by many authors [6, 10, 13, 15 and others]. The electrophoretic mobility of these proteins is close to the mobility of the proteins of the actomyosin complex.

During paper electrophoresis the fraction of water-soluble myofibrillary proteins (fraction T in our terminology) remained on the paper strip at the starting point or in its immediate proximity. During free electrophoresis the order of arrangement of the individual peaks on the electrophoregram of the uterine muscle proteins was changed.

SUMMARY

Proteins extracted by Weber's solution from the smooth muscle of the uterus do not possess the ability to interact with myosin preparations with the formation of actomyosin. This makes highly improbable the assumption of Snellman and Tenow on the presence of any appreciable amounts of free actin in 0.5 M KCL extracts of myometrium.

Investigations conducted by the method of paper electrophoresis demonstrate the presence of 3 fractions in the myometrium extracts with high ionic potential, viz., myoalbumin, heterogeneous myogen and actomyosin. The latter consists of a small quantity of actomyosin (undoubtedly bound with nucleoproteins) and water-soluble myofibrillar proteins (mainly tropomyosin and possibly Δ-protein). Likewise these investigations give no grounds for associating the peculiarity of the myometrial proteins with the high concentration of the "easily extractable" actin in the muscles of the uterus.

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