REVISTA ESPAÑOLA DE FISIOLOGIA, 34, 103-110. 1978

Significance of a New Isovolumetric Technique for the Study of the Myometrial Dynamics *in vitro*

J. Santafé, J. Segarra-Doménech and A. Quintana

Departamento de Farmacología
Facultad de Medicina
Bilbao (España)

(Received on March 14, 1977)

J. SANTAFE, J. SEGARRA-DOMENECH and A. QUINTANA. *Significance of a New Isovolumetric Technique for the Study of the Myometrial Dynamics* in vitro. Rev. esp. Fisiol., 34, 103-110. 1978.

Above usual values for active tension are measured on 10 uterine horns of rat under electric stimulation through an original isovolumetric technique. At the maximum lenght increase attained were 1.79 ± 0.2 kg/cm², with passive tension from 9-10 % total tension. These values are within the range for other smooth and striated muscles. The isometric techniques efficacy to record total uterine tension, as well as the application of isometric activity to the analyse of uterine dynamics are discussed.

In the last years, the analysis of some parcial aspects of the dynamics of different smooth muscles has been emphasized, especially the evaluation of the length-tension relationship and the characteristics of the passive tension, which are the base for one of the mechanisms regulating the muscular activity, the Frank-Starling mechanism.

Conceptually, there should be no important qualitative differences between the dynamics of the smooth and the striated muscles, because both are programmed to develop the same function: to contract. With this conceptual base, we have demonstrated in previous works that the fundamental mechanic property of the uterine muscle is the inverse force-velocity relationship, and that the contractility is one of its regulating mechanisms (14, 16, 17). As to the second mechanism, i.e., the length-tension relationship, its existence has been proved in different smooth muscles: taenia coli (1, 6, 10, 15), duodenum (11), tracheal muscle (20), vascular smooth muscle (5, 9, 18), uterine muscle (4), etc. The length-tension diagrams are qualitatively similar in all these muscles, but quantitatively the scarce maximum active tension of the uterus strongly contrasts (up to 18 times inferior to other smooth muscles). This discordance can only be explained admitting that the uterine muscle has less capacity to develop tension than other smooth muscles, or rather, that the usual techniques are unable to record all developed active tension or only register the activity of a limited number of uterine fibres. *A priori,* there seems to be no reasons justifying the first mentioned possibility; as to the second one, the length-tension relationship is usually analyzed by isometric techniques, which are valid for muscles where the fibres run longitudinally with regard to their main axis, but their usefulness is doubtful in the uterus, where the muscular wall consists of fibers arranged in spirals with different degrees of opening. Consequently, the strict evaluation of the contractile ability of the uterine muscle requires a methodology which must take into consideration its structure, physiological activity and function, which are all of them premises not fulfilled in the isometric techniques.

Since these technical aspects are of utmost importance for physiologists and pharmacologists, in this work we propose a new isovolumetric technique to analyse the length-tension, relationship in the rat uterus, the only techniques which, in our opinion, agree with the before mentioned structural and functional conditions.

Materials and Methods

The work was made on 10 uterine horns from Wistar rats, weighing between 180 and 200 g. In order to avoid the changing influence of the ovarian hormones on the mechanic activity of the muscles, the animals were ovariectomyzed, and 14 days later an oily suspension of estradiol benzoate (250 μg/kg) was intraperitoneally administered; the rats were sacrified 48 hours after the injection and both uterine horns were removed. This method garantizes that all muscles work exclusively under the influence of the estrogens. The muscular pieces were then sectioned so that their length at rest was always 2.5 cm. Then they were placed in an organ bath at a temperature of 36° C, perfused with a Ringer's solution (154 mM NaCl; 5.6 mM

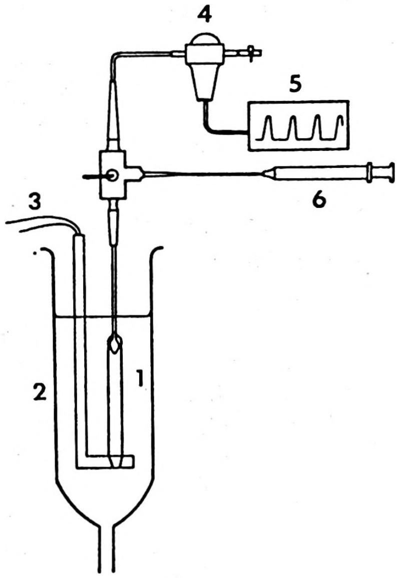

Fig. 1. *Diagram of the isovolumetric system.* 1. Uterine muscle; 2. Bath; 3. Electrodes; 4. Transducer; 5. Record system, and 6. Injector system.

KCl; 2.7 mM $CaCl_2$; 5.95 mM $NaCO_3H$; 5.5 mM glucose) and oxygenated by carbogen.

The muscles were stimulated electrically by means of trains of supraliminal square waves of 2 ms duration and 3 ms spaced; the duration of each train was 5 seconds.

The muscles worked under isovolumetric conditions, for which an original system designed, the diagram of which is shown in *figure 1:* the lower end of the muscle is fastened to a platform which has two silver electrodes; the upper end is connected to a catheter integrated in a system which, by means of a triple connection key, easily allows the communication of the uterine lumen with the outside (and optionally modify the intrauterine volume) or with a closed system where the recording system is integrated (pressure transducer Statham Model P23 Ser. and Beckman dynograph type Rs).

Experimental dynamics. Once the muscles had been stabilized, after making them contract for a minimum period of 60 minutes under above mentioned conditions, the smallest length of their fibres (initial length or l_0) was determined; for this purpose the muscles were induced to contract with the system open outside so that the liquid kept in their lumen was ejected until they were virtually empty. After reaching this point, the muscles were submitted to controlled length increases of their fibres, by the introduction of known volumes of Ringer's solution (2-5-10-15-20-30-40-65-90 μl) in the uterine lumen, recording the muscular activity in each one of the new lengths.

The measured parameters were: 1) Active pressure developed after the stimulation; 2) Passive pressure, secondary to the length increases of the muscular fibres.

Calculation of the wall tension. Considering the uterine horn as a cylinder, the wall tension was calculated through Laplace's law, knowing the pressure and the internal radius of the uterus (r_i). The wall thickness after each increase of the r_i is the difference between the external radius (r_e) and r_i. The r_e can easily be deduced from the cross-sectional area, calculated as follows:

$$A = \frac{M}{d \cdot L}$$

where: A, is the cross-sectional area (cm^2); M (muscular mass), was determined after having dried the muscles; its value was 0.077 ± 0.0033 g; d (density), its value was 1.05 g/cm^3; L (length of the muscular pieces), was kept constant during all the experiences: 2.5 cm.

The value calculated for A was 0.029± 0.0016 cm^2.

Results

The values calculated for the internal radius and the wall thickness of the uterus are summarized in table I.

Table I. *Values (Mean±Standard error) of the internal radius and the wall thickness according to the increases of the intrauterine volume.*

	Intrauterine volume (μl)	Internal radius (cm)	Wall thickness (cm)
l_0	0	0	0.096±0.0030
l_1	2	0.0159	0.081±0.0026
l_2	5	0.0252	0.074±0.0026
l_3	10	0.0355	0.066±0.0026
l_4	15	0.0437	0.061±0.0026
l_5	20	0,0504	0.058±0.0023
l_6	30	0.0618	0.052±0.0023
l_7	40	0.0713	0.048±0.0023
l_8	65	0.0909	0.041±0.0020
l_9	90	0.107	0.037±0.0016

The recorded active pressure increases sharply until the internal radius reaches the value corresponding to l_3, and then it decreases without interruption. The behaviour of the passive pressure is qualitatively different, its value for each increase of the internal radius being always superior to that of the preceeding length (table II).

The average of the 10 experiences shows that the wall active tension, calculated from the data shown in the preceeding tables, rises uninterruptedly during the successive increases of the internal radius: its value was 0.153±0.014 kg/cm^2 in l_1,

Table II. *Values (Mean±Standard error) of the active and passive pressures for the consecutive increases of the intrauterine volume.*

	Pressure (g) Active	Passive
l_0	0	0
l_1	22.72±2.00	0.19±0.02
l_2	29.07±2.00	0.29±0.03
l_3	31.12±2.26	0.48±0.04
l_4	29.87±2.42	0.56±0.05
l_5	28.28±2.62	0.64±0.06
l_6	26.06±2.67	0.87±0.11
l_7	24.65±2.48	1.11±0.10
l_8	20.91±2.27	1.44±0.09
l_9	18.68±2.21	1.82±0.08

Table III. *Behaviour of the wall tension (Mean ±Standard error) according to the intrauterine volume.*

	Tension (kg/cm²) Active	Passive
l_0	0	0
l_1	0.153±0.014	0.0013±0.0002
l_2	0.338±0.015	0.0036±0.0006
l_3	0.565±0.017	0.0096±0.0015
l_4	0.714±0.025	0.0149±0.0024
l_5	0.827±0.032	0.0210±0.0035
l_6	1.023±0.047	0.0387±0.0072
l_7	1.221±0.052	0.0610±0.0091
l_8	1.537±0.071	0.116 ±0.014
l_9	1.794±0.123	0.190 ±0.019

and reaches 1.794±0.123 kg/cm² at greatest uterine distension (table III).

The wall passive tension shows a similar behaviour; its value was 0.0013±0.0002 kg/cm² in l_1, rising up to 0.19±0.019 kg/cm² in l_9 (table III) However, this parameter increases proportionally more than the active tension, since when the latter in l_9 grew 11.7 times with regard to l_1, at the same point the passive tension was 13.8 times superior to the initial one; also the increases of tension after each increase of the internal radius were higher at passive tension.

The pressure and tension values shown in tables II and III, were submitted to the analysis of the differences test, and the increments have statistical significance ($p < 0.001$).

Figure 2 shows the evolution of the active and passive tension of one of the 10 uteri tabulated in table III.

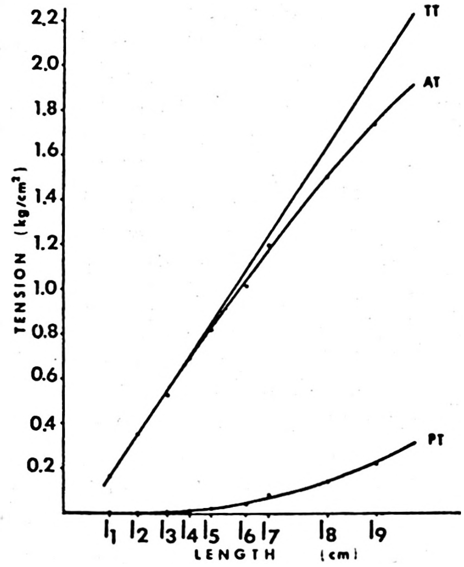

Fig. 2. *Length-tension diagram corresponding to one of the ten uterine horns included in table III.*
Legend: AT = active tension; PT = passive tension; TT = total tension (AT + PT).

Discussion

When analyzing the uterine length-tension diagrams, the small active tension contrasts strongly when comparing this parameter with that of other kinds of muscles (table IV). These differences are even more emphazised when the active tension is expressed exclusively referred to the fraction of the cross-sectional area corresponding to the muscular tissue: 83 % in the frog sartorius (2), from 55 % to 69 % in the vascular wall (8, 21), and 76 % in the cardiac muscle (12), although in the last muscle about 40 % of the muscular tissue consists of noncontractile material, mainly mitochondria (19). If the values of the table IV are corrected according to these data, it becomes evident that the smooth muscular fibre developes an active tension per surface unit, similar to that of the skeletal muscular fibre, and in both cases clearly higher than the cardiac muscle: the arterial smooth muscle (used as prototype), 2.4-3.5 kg/cm²; the frog sartorius, 2.4-2.9 kg/cm²; and the cat papillary muscle, 1.4 kg/cm². The differences persisting after these corrections may be explained by divergences in stimulation or temperature; because the cardiac muscle cannot be tetanized, etc.

However, the above considerations do not explain the low maximum isome-

Table IV. *Values of the maximum isometric tension in different kinds of muscles.*

	Maximum isometric tension (kg/cm²)	Reference
	2.3	(15)
Guinea-pig taenia coli	1.5	(10)
	1.82	(1)
Rabbit taenia coli	0.89	(6)
Cat duodenum	0.42	(11)
Dog tracheal muscle	1.17	(20)
Bovine mesenteric artery	2.1	(9)
Rabbit iliac artery	1.6	(18)
Dog carotid artery	1.1	(5)
Rabbit uterus	0.13	(3, 4)
Rat uterus	0.15	(Santafé *et al.*, unpublished)
Frog sartorius	2.1	(7)
	2.4	(19)
Rat diaphragm	1.0	(13)
Cat papillary	0.64	(19)

tric uterine tension, between 3 and 18 times inferior to that of other smooth muscles. With respect to its function there is no support for this fact, which can only be justified througth technical reasons. Usually, the length-tension relationship is analyzed by isometric techniques, which record the activity of the longitudinally working muscular fibres. Obviously, these techniques are valid for muscles such as the frog sartorius, taenia coli or papillar of cat, where it is possible to admit that all their fibres adopt the above mentioned arrangement; however, the utility is more doubtful in organs like the uterus, where the complex muscular layer is formed by spiral fibres, more or less open, but without strictly longitudinal or transversal fibres. When an isolated uterus is placed on an isometric device, with both ends fastened, and is stretched, all the fibres adopt a more vertical disposition (figure 3, A), but while the more longitudinal fibres are clearly stretched (*a-a'*), the length of the more transversal fibres increases to a much lower extent

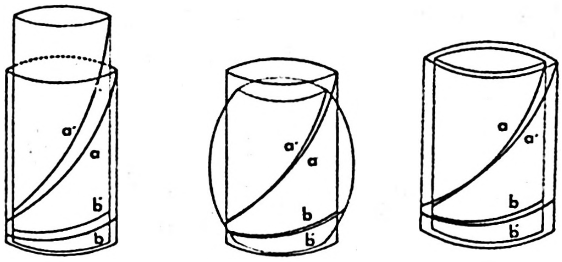

Fig. 3. *Changes of the length of the muscular fibres under different experimental conditions.*
(Explanation in the text). Legend: A = by usual isometric techniques; B = *in situ;* C = by our isovolumetric technique; a-a' = fibres in open spiral (essentially longitudinal); b-b' = fibres in closed spiral (essentially transversal).

(*b-b'*); i.e. not all fibres are stretched to the same degree. Moreover, the system can only record the activity of those fibres that work longitudinally, and subsequently most activity of the most transversal fibres is lost; this fact may explain the low maximum isometric tension recorded in the uterus.

On the other hand, physiologically the isometric activity does not exist in the uterus. The best model with which this organ can be compared is the heart, where it is universally accepted that the ventricles behave in diastole like a cylinder, but during the systole they tend to become spherical (figure 3, B): during the isovolumetric period of ventricular activity the most longitudinal fibres are shortened, whereas the most transversal fibres are stretched; i.e. the volume of the ventricular cavity is not modified, but changes the length of the fibres and subsequently according to international agreement, the term *isometric activity* has been replaced by *isovolumetric activity.*

According to the preceeding considerations there is no reason for maintaining, with reference to the isolated uterus, the usual terminology and study techniques, which should be replaced by the isovolumetric ones.

In the technique proposed in this study, any increase of the intrauterine volume will determine a proportional increase of fibre lengths, regardless of the degree of opening of the spirals (figure 3, C). Only by increasing the intrauterine volume it is possible to stretch all the fibres that form the wall and only by recording the intrauterine pressure it is possible to record the vector resulting from the activity of all fibres. This conceptual reasoning is supported by the results of the present work: figure 2 shows a typical length-tension diagram and the values of the active tension, as from 1.8 kg/cm^2 for the maximum distension reached (table III), can be compared with those developed by any of the previously analyzed muscles.

Only a part of the ascending limb of the length-tension diagram was recorded since the distension is an adequate stimulus for the uterine activity, so that the higher stretchings could determine a certain degree of activation, which might interfere with the results, especially those of the passive tension. This limitation is usually avoided by proceedings like causing a muscular supply insuficience by means of metabolic inhibitors (5); working at low temperatures (6); lowering the Ca^{++} concentrations, etc. In any case, these conditions of muscular activity are never the physiological ones, and the data attained using such techniques, reflect the muscular activity in a definite situation, which is also a limitation and may justify the disparity of data on the passive tension developed when increasing the muscular length. According to our results, the passive tension of the uterus assumes an important fraction of the total wall tension (9.5 % in l_o).

In conclusion, the results of this work show that the isovolumetric technique is the most adequate for studying the activity of the uterine muscle, and must be used in any study where the tension developed by the uterus is the parameter to be evaluated.

Resumen

En 10 cuernos uterinos de rata estimulados eléctricamente, mediante una técnica isovolumétrica original, se miden valores de tensión activa superiores a los habituales: 1,79±0,12 kg/cm^2 para el máximo incremento de longitud alcanzado; en este punto, la tensión pasiva es el 9-10 % de la tensión total. Estos valores están dentro de la escala de los señalados para otros músculos lisos y estriados. Se discute la eficacia de las técnicas isométricas para recoger la tensión uterina en su totalidad, así como el concepto de actividad isométrica aplicado al análisis de la dinámica uterina.

References

1. ABERG, A. K. and AXELSSON, J.: *Acta Physiol. Scand.*, **64**, 15-27, 1965.
2. BOZLER, E.: *J. Gen. Physiol.*, **50**, 1459-1465, 1967.
3. CSAPO, A.: *Am. J. Physiol.*, **177**, 348-354, 1954.
4. CSAPO, A. and GOODALL, M. C.: *J. Physiol., (London)*, **126**, 384-395, 1954.
5. DOBRIN, P. B.: *Am. J. Physiol.*, **225**, 664-670, 1973.
6. GORDON, A. R. and SIEGMAN, M. J.: *Am. J. Physiol.*, **221**, 1243-1249, 1971.
7. HILL, A. V.: *Proc. Roy. Soc. London, Ser. B*, **126**, 136-195, 1938.
8. JONES, A. W., FEIGL, E. D. and PETERSON, H. L.: *Circulation Res.*, **15**, 386-392, 1964.
9. LUNDHOLM, L. and MOHME-LUNDHOLM, E.: *Acta Physiol. Scand.*, **68**, 347-359, 1966.
10. MASHIMA, H. and HANDA, M.: *J. Physiol. Soc. Japan*, **31**, 627-628, 1969.
11. MEISS, R. A. and PROSSER, C. L.: *Fed. Proc.*, **28**, 712, 1969.
12. PAGE, E. and SOLOMON, A. K.: *J. Gen. Physiol.*, **44**, 327-344, 1960.
13. RITCHIE, J. M.: *J. Physiol. (London)*, **123**, 633-639, 1964.
14. SANTAFÉ, J.: Tesis Doctoral. Valencia, 1971.
15. SCHATZMANN, H. J.: *Pflüger's Arch. ges. Physiol.*, **274**, 295-310, 1961.
16. SEGARRA-DOMÉNECH, J., SANTAFÉ, J., ORTS, A., VILA, J. and BEDATE, H.: *XII Reun. Nac. Soc. Esp. C. Fisiol.*, Santiago de Compostela, 1970, pp. 139-141.
17. SEGARRA-DOMÉNECH, J., SANTAFÉ, J., ORTS,

A., VILA, J. and ESPLUGUES, J.: *Proc. Intern. Union Physiol. Sci.*, **9**, German Physiological Society. Munich, 1971, p. 147.

18. SOMLYO, A. P. and SOMLYO, A. V.: *Pharmacol. Rev.*, **20**, 197-221, 1968.
19. SPIRO, D. and SONNEBLICK, E. H.: *Circulation Res.*, **15**, suppl. II, 14-37, 1964.
20. STEPHANS, N. L., KROEGER, E. and MEHTA, J. A.: *J. Appl. Physiol.*, **26**, 685-692, 1969.
21. VILLAMIL, M. F., RETTORI, V., BARAJAS, L. and KLEEMAN, C. R.: *Am. J. Physiol.*, **214**, 1104-1112, 1968.