Isometric Contractions of Isolated Rat Uterine Muscle: Relation to Estrous Cycle and Pregnancy[1] (37434)

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The mechanical response of mammalian uterine muscle varies as its hormonal influence changes from estrogen to progesterone dominance. This can be seen in the increase in rate and amplitude of contractions of the uterus and fallopian tubes during the estrogen-dominated proestrus and estrus stages of the cycle in some species (1–4). Diminution in these parameters occurs in the postovulatory phase and in pregnancy, which are progesterone dominated. With the decline in progesterone levels towards the end of pregnancy, recently demonstrated in humans, mechanical activity increases and persists postpartum (5).

In rabbit uterine muscle strips obtained from oophorectomized animals pretreated with estrogen, Csapo and Corner (6) demonstrated a positive staircase phenomenon (increased developed tension with increased frequency of electrical stimulation). In those animals pretreated with estrogen and progesterone, the staircase became negative. This effect has been demonstrated in the rat by Coutinho (7) who also showed the dependence of the staircase on external Mg^{2+} concentration and suggested substitution of membrane Ca^{2+} by Mg^{2+} as its cause.

There are, however, no detailed studies on changes occurring in the isometric myogram of uterine strips obtained during the natural estrous cycle and pregnancy. The present report was designed to provide this information.

Methods. Twenty-one virgin adult white female Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing approximately 200 g (range 175–230 g) were used. Five 7 to 14 day pregnant rats of the same weight were also studied. Muscles which exhibited spontaneous activity which could not be prevented by bath changes and temperature regulation were omitted from analysis. This was observed in an additional 10 specimens.

Prior to decapitation, vaginal smears were obtained with a swab moistened with normal saline and the cells were examined in a saline drop at 100× magnification. The rats were typed into stages of the estrous cycle according to the histology of the vaginal smear and the macroscopic appearances of the vagina and uterus (8).

Although we separated these muscles into different stages, it must be remembered that the cycle is a continuous one and each stage merges into the next. Where there was doubt about the staging of a cycle, or if an intermediate stage was encountered, that muscle was not used. Care was taken not to induce a pseudo-pregnancy by rough handling when obtaining the vaginal smear and the rats were never examined more than once a day (9).

Rats are nocturnal breeders. Natural estrous begins in most cases in the late evening (10) and has passed by the following morning when we conducted our experiments. Accordingly, few of the nonpregnant muscles studied came from animals in estrus, and insufficient data was collected from this group. Diestrus muscles failed to stabilize in the muscle bath on a number of occasions, and were finally excluded from the study. Thus, proestrus, metestrus I, metestrus II and

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pregnancy are the groups reported in this study.

After sacrifice, one uterine horn was quickly excised and a strip of muscle was obtained from midway along the antemesometrial border. Each strip was trimmed to approximately the same length and cross-sectional area and suspended between two ring clamps in series with a micrometer. The muscle was induced to contract once each minute with a 15 Volt rms 60 Hz ac stimulus applied via two 1 cm^2 platinum electrodes arranged on either side of the muscle. Isometric tension was detected with a tension transducer (Statham UC-3), amplified, displayed on an oscillographic recorder, and stored on magnetic tape for future reference.

The muscle strip was continually bathed in Krebs–bicarbonate solution, the composition of which in mEq/liter was: Na^+, 146; K^+, 5.0; Ca^{2+}, 4.5; Mg^{2+}, 2.5; Cl^-, 130; HCO_3^-, 25; HPO_4^{2-}, 1.4; SO_4^{2-}, 2.5. The solution also contained 11.1 m*M* glucose and 0.026 m*M* EDTA. A 95% O_2–5% CO_2 mixture was bubbled through the bath at 5 cc/min and the pH was maintained at 7.4. The bath was rapidly emptied and refilled with fresh solution at 15 min intervals. Temperature was controlled via a coil around the muscle bath containing circulating water from a refrigerated reservoir, and was maintained at 20 ± 1°.

Each muscle was allowed to equilibrate for 30 min with a resting tension of approximately 0.25 g. A length tension curve was then undertaken with length increases of 0.5 mm. Sufficient time (5–10 min) was allowed between each length increase to permit stabilization of stress relaxation to an asymptote. Each muscle was studied at the length where developed tension reached a plateau value, and the resting tension had begun to increase steeply. After an additional 20 min of contractions at this length, one twitch was recorded at a paper speed of 2 mm/sec.

At the conclusion of each experiment, the muscles were dried overnight and then weighed. As the density of uterine muscle varies during the normal stages of the estrous cycle (11), it was not possible to calculate wet cross-sectional area directly. An estimate of the mass of formed elements per unit length of the specimens was gauged by assuming a constant arbitrary density of the dried muscle strips of 1 g/cm^3.

The dry muscle strip cross-sectional area was calculated from the formula

$$\text{EXSA} = \frac{W}{D \times L},$$

where EXSA = estimated dry cross-sectional area (cm^2).

W = dry weight (g),

D = density of dried muscle (g/cm^3), and

L = muscle length (cm).

In the isometric myogram, peak developed tension (Tpd), the greatest amount of isometric tension developed over resting tension (Tr) after a stimulus, corrected for EXSA, was measured. The time to peak tension (TPT) was taken as the time from the onset of the stimulus until Tpd. Each myogram was integrated to obtain the tension time integral (TTI) which was corrected for EXSA. Relaxation times were measured at two points: the time for Tpd to decline to 50% (RT_{50}) and to 90% (RT_{90}) of maximum.

The statistical method employed was that of analysis of variance with $p < 0.05$ taken as the level of significance.

Results. The general contours of the myograms were found to be reproduceable from muscle to muscle within each group. Certain differences were noted between the groups, however (Fig. 1). In those muscles considered to be progesterone-dominated (metestrus I and pregnant), tension rose during the stimulus to near maximal levels. After discontinuation of the stimulus, tension smoothly tapered to a peak. The estrogen-dominated muscles (proestrus and metestrus II) demonstrated a biphasic tension development: an initial phase during the stimulus followed by a secondary surge of tension development. The second phase always began immediately after the stimulus ended, and was not altered by modifying the duration or strength of the stimulus.

Neither the Tpd nor the TTI were affected by the stage of the estrous cycle (Table I).

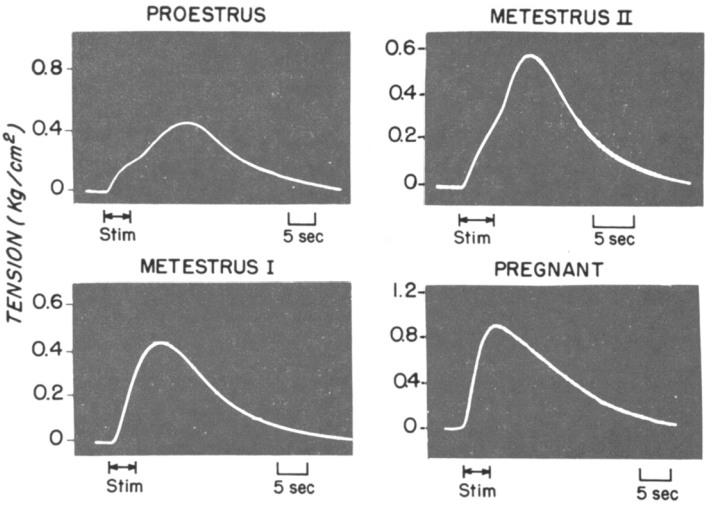

FIG. 1. Isometric myograms showing characteristic twitch configurations for each group. Note shorter contraction and relaxation times in the metestrus I and pregnant examples, and greater developed tension in the pregnant illustration. Stim = stimulus.

However, in the pregnant muscles, Tpd was increased 127% ($p < 0.001$) and TTI was increased 80% ($p < 0.05$) over the means of the nonpregnant groups.

TPT significantly shortened with decreasing estrogen influence in the nonpregnant groups ($p < 0.01$). The TPT in pregnancy was not significantly different from that of metestrus I but was 70% greater in the estrogen-dominated groups compared with the progesterone-dominated groups ($p < 0.001$). The difference between proestrus and metestrus II was not statistically significant.

Relaxation times were prolonged in the estrogen-dominated groups. Mean RT_{50} was 60% longer ($p < 0.001$) and mean RT_{90} was 37% longer ($p < 0.001$) in the estrogen-dominated, as compared to the progesterone-dominated groups. Pregnancy did not alter RT_{50} or RT_{90} when compared with metestrus I.

There were no significant differences among the EXSA of the nonpregnant groups, but the trend to a smaller mean EXSA in the proestrus group is compatible with Astwood's

TABLE I. Mechanical Properties of Uterine Muscle Strips Obtained at Different Stages of the Estrous Cycle and Pregnancy.[a]

	Estrogen-dominated		Progesterone-dominated	
	Proestrus	Metestrus II	Metestrus I	Pregnant
Tpd (kg/cm^2)	0.48 ± 0.05	0.46 ± 0.10	0.54 ± 0.10	1.13 ± 0.24
TTI ($kg\text{-}sec/cm^2$)	4.7 ± 0.5	4.25 ± 0.92	3.08 ± 0.62	7.21 ± 1.87
TPT (sec)	16.8 ± 1.3	11.8 ± 0.7	9.16 ± 0.77	7.55 ± 0.38
RT_{50} (sec)	15.7 ± 2.0	15.6 ± 2.2	8.8 ± 1.4	10.9 ± 0.6
RT_{90} (sec)	32.4 ± 1.8	34.4 ± 3.0	23.9 ± 2.2	24.6 ± 3.0
EXSA ($cm^2 \times 10^{-3}$)	1.57 ± 0.13	2.35 ± 0.17	1.93 ± 0.20	1.98 ± 0.19

[a] The stages of the estrous cycle have been arranged with metestrus II interposed between proestrus and metestrus I so that the estrogen-dominated groups are to the left and the progesterone-dominated groups to the right, even though chronologically metestrus II follows metestrus I. All data are means ± standard errors. Tpd = peak developed isometric tension; TTI = tension time integral; TPT = time to peak tension; RT_{50} = 50% relaxation time; RT_{90} = 90% relaxation time; EXSA = estimated cross sectional area.

finding of maximal hydration of the uterus during this phase (11).

Discussion. A continuous estrogen influence is present in varying degrees throughout the reproductive life of the female mammal. Progesterone dominance occurs only if this hormone is present above a critical concentration (12).

At the beginning of proestrus in the rat, progesterone levels are low (13). Estrogen, which begins rising in late diestrus, reaches a peak during proestrus (14). This causes a surge of pituitary gonadotropins, which in turn stimulates progesterone secretion from the ovary. As progesterone approaches a peak in late proestrus, the estrogen concentration rapidly declines. Spontaneous ovulation occurs during estrus when both hormones have passed their maximum concentrations, but when significant progesterone effect is still present. In females kept apart from males, levels of both ovarian hormones then decrease rapidly as a spurious corpus luteum is formed (1, 10).

Based on these considerations, the proestrus muscles in this study were judged to be estrogen dominated. Confirmatory evidence of this was found in progressive uterine intraluminal fluid collection, which is an estrogen-induced phenomenon. During pregnancy and metestrus I, muscles were considered to be under both estrogen and progesterone effect, but progesterone dominated. Because progesterone is absent during metestrus II, these muscles were considered estrogen dominated.

Thus, proestrus and metestrus II were considered estrogen dominated, and pregnancy and metestrus I were considered progesterone dominated.

The major differences in the isometric myograms of these groups were the longer contractions and relaxation times of the estrogen-dominated muscles compared to those under progesteron influence. Similar results were observed in uterine strips obtained from oophorectomized rabbits pretreated with estrogen or estrogen plus progesterone (6).

Within the estrogen- and progesterone-dominated groups, there was little variation in both the time to peak tension and the relaxation times. Since pregnant muscles are under much more progesterone influence than metestrus I, this suggests that a maximal effect on the time parameters of the isometric myogram is exerted by a critical estrogen–progesterone ratio, and that this may be an all or none effect.

Pregnant muscles generated greater developed tension than the nonpregnant ones, which is to be expected in view of the physiological demands of the pregnant uterus. Our pregnant animals were in the midthird of pregnancy when a significant amount of uterine distention had occurred. This distention of the myometrium by the growing conceptus is a potent stimulus for actin and myosin production and hence tension generation potential (15).

The "blocking" action of progesterone confers a number of safety devices against premature labor. Foremost among these are the diminished excitability of the cell membrane (16–18) and the negative staircase phenomenon (7). The shortened contraction and relaxation times demonstrated in this study would also act to lessen the cervix-dilating effect of any contraction.

As the onset of labor approaches, the progesterone block weakens and the pattern of myometrial activity alters towards that of estrogen domination. This may be observed *in vivo* as an increasing frequency of contraction (increased excitability) and increasing tension with each contraction (positive staircase phenomenon). The longer contraction and relaxation times of the estrogen dominated muscle together with the increased contractile potential of the pregnant muscles (as manifested by their enhanced Tpd and TTI) combine to provide the necessary cervix-dilating force required for efficient labor.

In the nonpregnant state, the cycle of estrogen or progesterone domination occurs and is related to ovum transport and implantation. In this regard, increased motility is required during the estrogen-dominated stages to facilitate ovum capture and transport by the uterus and fallopian tube. Relative quiescence of the myometrium is also necessary in the progesterone-dominated (postovulatory) stages to permit implanta-

tion of the embryo. These changes in function are modulated by the same factors operative during pregnancy and labor. However, no change in developed tension is necessary in the nonpregnant state for these activities and none was demonstrated in the present experiments. This suggests that the range of estrogen concentrations during the natural estrous cycle is insufficient to significantly alter the amount of actin and myosin. Alternatively, there may be insufficient duration of maximal estrogen concentration to permit enough growth of contractile protein to significantly alter developed tension as measured with our apparatus.

In summary, it has been shown that the contraction and relaxation times of estrogen-dominated rat myometrium (proestrus and metestrus II) are prolonged compared with progesterone-dominated myometrium (pregnancy, metestrus I). The pregnant myometrium generates much more tension than either estrogen or progesterone-dominated nonpregnant muscle. It is suggested that the changes observed in contraction patterns are consistent with the evolution of uterine muscle function during pregnancy and labor.

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