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Characterization of late-pregnant rat uterine contraction via the contractility ratio in vitro Significance of α_1-adrenoceptors

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Abstract

The aim of this study was to characterize the ability of late-pregnant (days 15–22) rat uterine tissue rings to contract in response to electric field stimulation *in vitro*. For this purpose, maximum rhythmic contractions were elicited by optimum choice of the period time and the pulse width, the two main parameters of electric field stimulation. In parallel, the plasma 17β-estradiol and progesterone levels were determined. It was found that the contractility ratio, i.e. the quotient of the optimum pulse width and the period time, is a good parameter with which to express the contractility. The larger the contractility ratio, the better the ability to contract. Evaluation of the area under the curve did not furnish information relating to the contractility in this method. A very close correlation was observed between the contractility ratio and the quotient of the 17β-estradiol and progesterone levels on different days, demonstrating that the *in vitro* ability characterized by the contractility ratio is in keeping with the physiological regularity. There was also a very close correlation between the contractility ratio and the quotient of the α_1- and β-adrenergic receptors, suggesting the main role of the numbers of α_1-receptor in pregnant uterine contractility. It is believed that this is the first *in vitro* model to give a numerical measure concerning the ontogeny of uterine contractility in late pregnancy.

Keywords: Uterine contractility; Electric field stimulation; Sex hormones; Late pregnancy; α_1-adrenoceptors

Introduction

During pregnancy, the uterus undergoes a maturation process resulting in labour pains and delivery. This complex mechanism is controlled by a heterogeneous regulation, involving

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physiological factors such as oxytocin, the adrenergic system, prostaglandins, connexin and sex hormones [1–3]. Animal investigations of uterine contractility in late pregnancy are essential in research relating to the suppression of premature labour, but such investigations are difficult. During pregnancy, *in vivo* study techniques such as the Millar cathether method of Csapo [4] are not suitable in the most frequently used animal models (rat, guinea pig and rabbit). *In vitro* contractility studies are another possibility, and are accepted for electric field stimulation (EFS). The few such studies performed so far have concentrated on the excitability of the uterine muscle through the nerves [5–7] or the changes in response to electric stimuli in the presence of various drugs [8–11]. However, such investigations are not able to characterize the ontogeny proceeding in the contractility of the uterus in late pregnancy.

The aim of the present study was to characterize the ability of such uterine tissues to contract in response to EFS in late pregnancy (days 15–22) in the rat. For this purpose, we set out to describe the contractility by choosing stimulation parameters necessary to elicit rhythmic contractions. Additionally, we searched for a correlation between the change in contractility *in vitro* and the plasma levels of sex hormones, in the interest of a comparison of our results with physiological principles. Finally, we determined the changes in density of the adrenergic receptors in order to clarify their contributions to the contractility response of the late-pregnant rat uterus.

Methods

Uterus preparation and EFS

Uteri were removed from Sprague-Dawley rats (250–350 g) on day 15, 18, 20 or 22 of pregnancy. Muscle rings 0.5 cm long were sliced from the uterine horns and mounted vertically between two platinum electrodes in an organ bath containing 10 ml de Jongh solution (in mM: 137 NaCl, 3 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 12 $NaHCO_3$, 4 NaH_2PO_4, 6 glucose, pH: 7.4). The organ bath was maintained at 37 °C and carbogen (95% O_2 + 5% CO_2) was bubbled through it. After mounting, the rings were equilibrated for about 1 h before experiments were undertaken. The initial tension was set to about 1.25 g, which was relaxed to about 0.5 g at the end of equilibration. Maximum rhythmic contractions were elicited with a digital, programmable stimulator (ST-02, Experimetria U.K. Ltd.), using different values of pulse width (PW, the duration of the electric field as a single stimulus) and period time (PER, the time interval between two stimuli) at 40 V for 240 s. The shortest interval time was sought with which to elicit rhythmic contractions. After identification of this value, the pulse width was gradually increased as much as possible at the constant time interval to maintain the rhythmic contractions. The tension of the myometrial rings was measured with a gauge transducer (SG-02, Experimetria U.K. Ltd.) and recorded with an ISOSYS Data Acquisition System (Experimetria U.K. Ltd.). Areas under curves (AUCs) and correlations were evaluated and were analysed statistically with the Prism 2.01 (GraphPad Software, USA) computer program. For statistical evaluations, data were analysed by ANOVA Neuman-Keuls post-hoc test.

Determination of plasma 17β-estradiol and progesterone

Blood samples were collected by cardiac puncture immediately before removal of the uteri. After centrifugation, the plasma was separated and stored at −20 °C until determina-

tion. 17β-Estradiol and progesterone were determined by radioimmunoassay. Reagent kits were purchased from the WHO Matched Reagent Programme. The lower limits of the 17β-estradiol and progesterone determinations were 30 pM and 0.5 nM, respectively. No cross-reaction was found in either case. The intraassay and interassay coefficients of variation for 17β-estradiol were 7.5% and 14.3%, and those for progesterone were 5.2% and 12.7%, respectively. Statistical analysis was carried out as mentioned above.

Radioreceptor binding assays

Radioligand binding experiments were carried out on the membrane preparation of pregnant rat uterus. The preparation was conducted as described by Maltier and Legrand [12].

The reaction mixture contained 100 μl membrane preparation (∼0.5 mg/ml protein), 100 μl tritiated ligand and 100 μl unlabelled ligand for non-specific binding or 100 μl incubation buffer (consisting of 0.05 M Tris-HCl, 0.01 M $MgCl_2$ and 2.5% ethanol pH = 7.42) for total binding. Incubations were started by addition of the membrane suspension and continued in a shaking water bath until a steady state was achieved (37 °C, 10 min for α-adrenoceptors and 30 °C, 30 min for β-adrenoceptors). At the end of the incubation, the bound radioligand was separated from the residual free radioligand by rapid filtration on a Brandell cell harvester through Whatman GF/C filters and washed with 3 × 10 ml of ice-cold buffer (Tris-HCl, pH= 7.42). The bound radioactivity was determined in a HighSafe scintillation cocktail in a Wallac 1409 liquid scintillation counter.

Saturation analysis of α_1-adrenoceptors was performed by incubating membranes with 0.1–2.5 nM [^{3}H]prazosine with or without 10 μM unlabelled phentolamine. In the case of β-adrenoceptors, the saturation analysis was performed with 0.25–10 nM [^{3}H]dihydroalprenolol in the presence or absence of 1 μM unlabelled propranolol.

Specific binding was determined by subtracting the non-specific binding from the total binding. All assays were carried out at least three times in duplicate and values are given as means ± SEM. In the investigated tissues, the K_d values are 0.48±0.05 and 1.87±0.27 for ^{3}[H]prazosine and ^{3}[H]dihydroalprenolol, respectively. Statistical analysis was carried out as mentioned above.

Results

The maximum rhythmic contractions elicited by EFS in uterine rings sliced out on pregnancy day 15, 18, 20 or 22 are illustrated in **Figure 1 a-d**. There were no significant differences in the average AUCs relating to the contractions (**Figure 1 e**).

Different PWs and PERs were used for *in vitro* electric field stimulation to elicit maximum rhythmic contractions. The contractility ratio PW/PER was significantly greater on day 18 than on day 15; there was a significant change between days 18 and 20, and the ratio was markedly elevated on day 22 (**Table 1, Figure 2**).

There were changes in the levels of plasma 17β-estradiol and progesterone between days 15 and 22. There were no significant changes in the ratio of 17β-estradiol and progesterone (E/P) between days 18 and 20, only a slight decrease was observed on day 20, but there was a sudden increase on day 22 (**Table 2, Figure 3**).

The maximum numbers of binding sites (B_{max}) for the α_1-receptors and β-receptors also

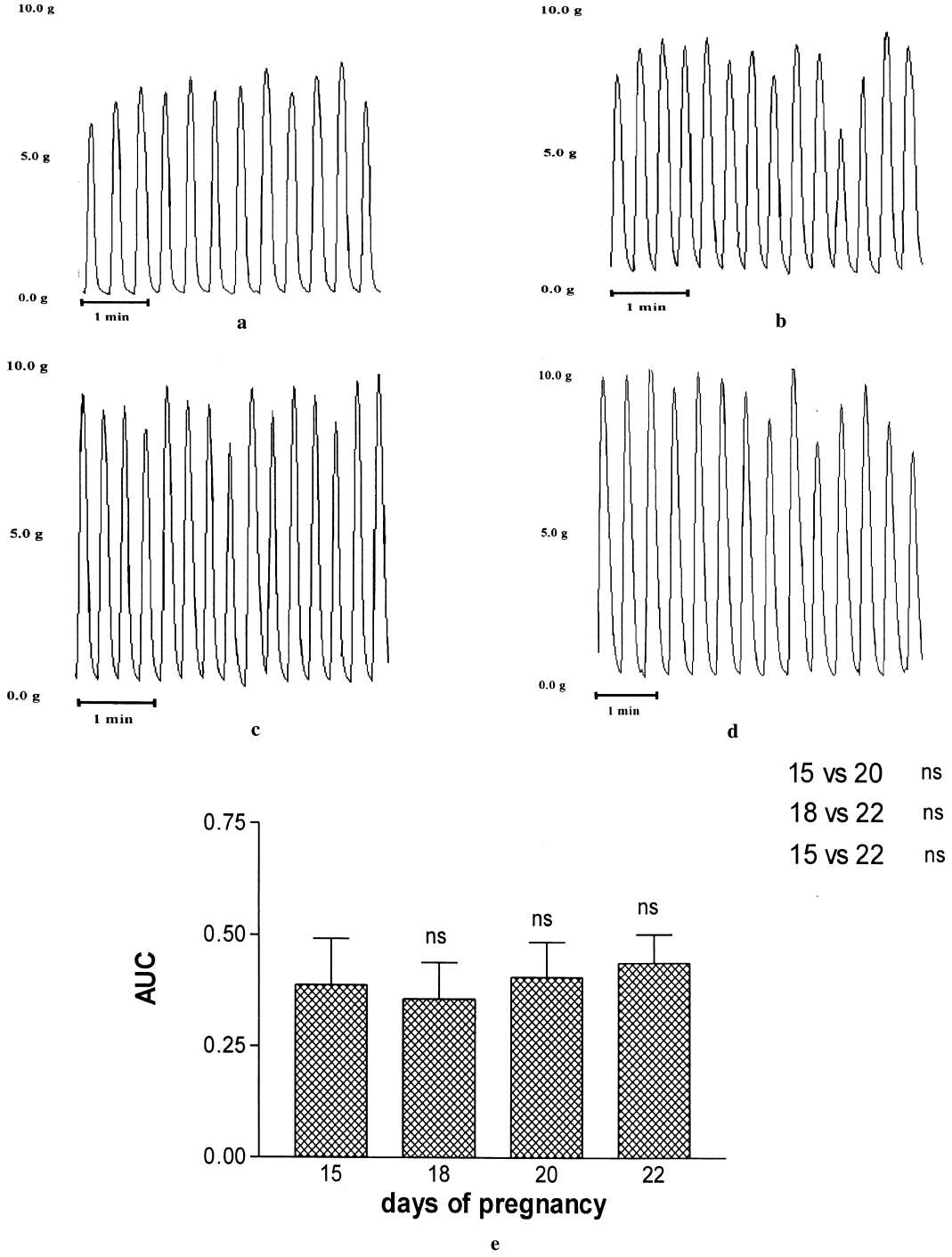

Fig. 1. Representative maximum rhythmic contraction curves elicited by electric field stimulation on day 15 (a), 18 (b), 20 (c) or 22 (d) of pregnancy *in vitro*, and average areas under the curves of maximum rhythmic contractions (e) on days 15–22 of pregnancy (ns=not significant).

Table 1
The pulse widths and period times for *in vitro* electric field stimulation eliciting maximum rhythmic contractions in late-pregnant rat uterine rings (n=8)

	Days of pregnancy			
EFS parameters	15	18	20	22
PW (ms)	60.00 ±10.00	75.00 ± 11.18	62.50 ± 12.50	150.00 ± 28.87
PER (s)	23.00 ± 1.22	18.33 ± 1.67	17.50 ± 1.44	23.75 ± 3.75

EFS=electric field stimulation, PW=pulse width [ms ± S.E.M.], PER=period time [s ± S.E.M.]

changed during the examined period. The ratio of α_1- and β-adrenergic receptors (α_1/β) was significantly higher on day 18; there was a non-significant, but slight decrease between days 18 and 20. There was an increase on day 22 (**Table 3, Figure 4**).

Regression analysis revealed very close correlations between PW/PER and E/P (**Figure 5 a**), and between PW/PER and α_1/β (**Figure 5 b)** ($r^2 = 0.910$ and 0.949, respectively) on the different days. A weaker correlation was found between E/P and α_1/β ($r^2 = 0.797$) (**Figure 5 c**).

Discussion

Research on pregnant uterine contractility is essential for an understanding of the processes that occur during delivery, and for the possibility of influencing these processes. We sought an animal model suitable for investigations of the ontogeny of contraction (the maturation process of the pregnant myometrium, expressed as the changes in the ability to contract toward term), in which changes are detectable during short periods of time and where there is an estrogen dominance similarly to that in humans at the end of pregnancy. Accordingly, we conducted experiments on pregnant rat: this offers a good model, with a 22-day period of pregnancy and a relatively high plasma estrogen level at term [13].

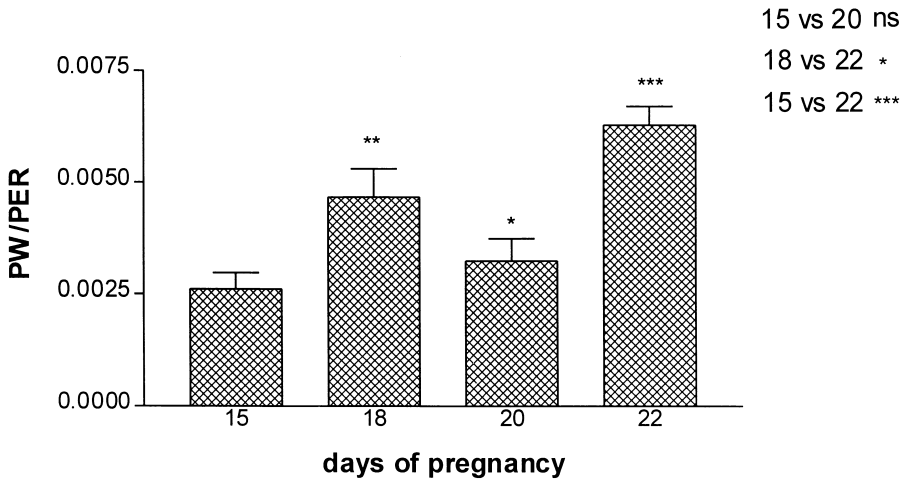

Fig. 2. Changes in contractility ratio (PW/PER) during late pregnancy in the rat (ns=not significant, *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$).

Table 2
The plasma 17β-estradiol [pM ± S.E.M.] and progesterone [nM ± S.E.M.] levels on different days of pregnancy in the rat (n=7)

	Days of pregnancy			
Sex hormones	15	18	20	22
17β-estradiol (pM)	251.13 ± 23.42	420.05 ± 32.82	244.20 ± 85.48	495.00 ± 54.73
progesterone (nM)	269.54 ± 38.52	168.18 ± 12.62	148.10 ± 18.44	26.10 ± 13.14

EFS is an accepted method for *in vitro* investigations of the uterus. The two important parameters of EFS are PW and PER. PW determines the stimulation of the smooth muscle through the nerve elements (<5 ms) or directly (>50 ms) [14]. In our investigations, direct uterine stimulation was applied, with regard to the fact that a nerve element denervation process can be detected at the end of pregnancy in rats [15]. Following the supramaximal stimulation rule [16], 40 V was used as stimulus voltage.

The physiological contractions of the pregnant uterus are usually rhytmic, and we therefore tried to elicit such contractions. They were considered rhythmic if the isolated uterine ring described a full contraction curve within one PER without a significant change in baseline tone. The shorter the PER that can be applied, the better the tissue contractility. PW has an important role in producing rhythmic contractions. With regard to the denervation process in the late-pregnant uterus, the applied PW was =50 ms. Such a stimulus opens the voltage-dependent Ca^{2+} channels and starts an influx of Ca^{2+} ions from the extracellular space to the intracellular space, but does not alter the Na^{+} channels of the nerves. The background of this selectivity is that the smooth muscle has a much longer time constant (60–133 ms) as compared those with of the nerves (0.7–5.0 ms). The short stimuli therefore open the Na^{+} chan-

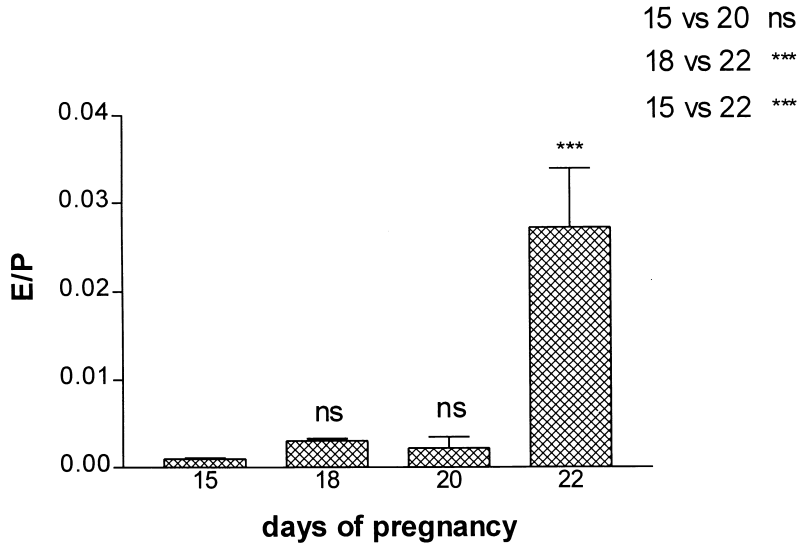

Fig. 3. Changes in the ratio 17β-estradiol/progesterone (E/P) during late pregnancy in the rat (ns=not significant, ***=p<0.001).

Table 3
Maximum numbers of binding sites (B_{max} ± S.E.M) of the adrenergic receptors on different days of pregnancy in the rat (n=7)

	Days of pregnancy			
Receptor types	15	18	20	22
α_1-adrenoceptor (fmol/mg protein)	344.93 ± 10.16	420.05 ± 32.82	244.20 ± 85.48	495.00 ± 54.73
β-adrenoceptor (fmol/mg protein)	15.99 ± 0.81	9.57 ± 1.62	7.21 ± 1.11	9.68 ± 0.82

nels in the nerves, while stimuli greater than 50 ms selectively open the Ca^{2+} channels of the smooth muscle [7]. The smooth muscle has no significant intracellular Ca^{2+} store. Although sarcoplasmatic reticulum and ryanodine receptors are known in the myometrium, their roles in smooth muscle contractile activation remain poorly understood [17,18]. The best known uterine contracting agents, e.g. oxytocin and prostaglandins, lose their effects after removal of the extracellular Ca^{2+} [19]. The smooth muscle contraction therefore depends greatly on the amount of extracellular Ca^{2+} ions entering the cell. Accordingly, a longer PW allows a higher concentration of Ca^{2+} ions in the uterine smooth muscle cell, which can result (within a given limit of approximately 50–200 ms) in stronger contractions. The longer the optimum PW, therefore, the more difficult it is to describe a full contraction curve within one PER. Thus, the longer the PW, the better the contractility. The elicited rhythmic contractions are referred to as maximum because the stimulation parameters were chosen in such a way that the tissues were not able to describe rhythmic contractions, or the AUCs were not higher for shorter PER or longer PW values.

AUC evaluation is an accepted method of comparing contraction curves. We determined the optimum stimulation parameters for maximum contractions via the AUCs. However, we

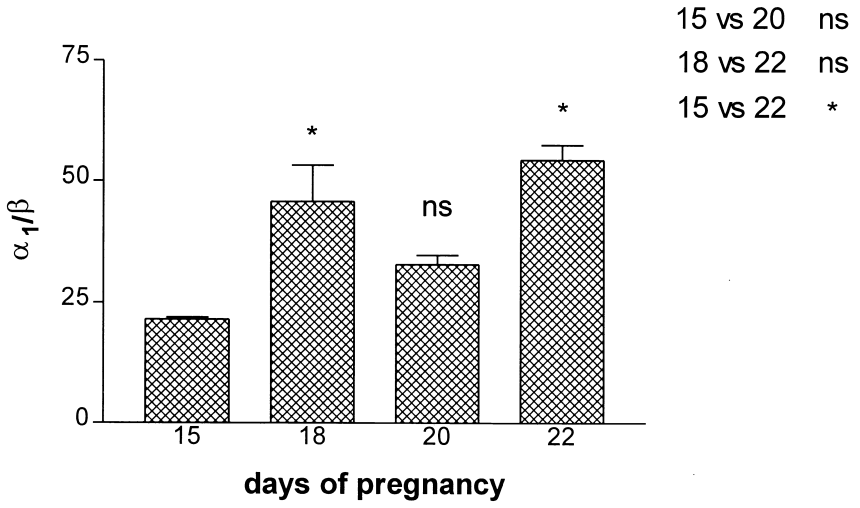

Fig. 4. Changes in the ratio of α_1- and β-receptor densities (α_1/β) during late pregnancy in the rat (ns=not significant, *=$p<0.05$).

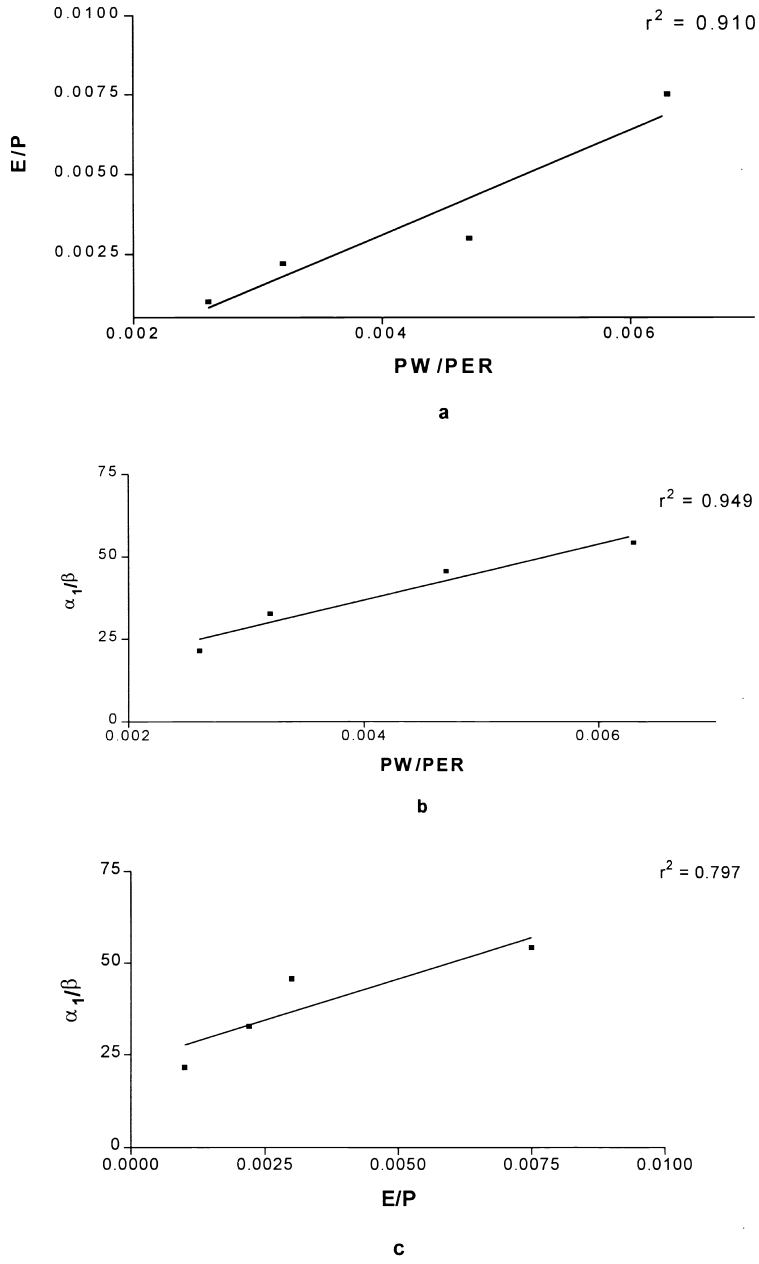

Fig. 5. Correlations between the contractility ratio (PW/PER) and the ratio 17β-estradiol/progesterone (E/P) (a), between PW/PER and the ratio α_1/β-adrenoceptor (α_1/β) (b), and between E/P and α_1/β (c) in the late-pregnant rat.

could not deduce the ability of uterine tissue rings to undergo contraction from the AUC values because there were no significant differences between the AUCs on the different days of pregnancy. The contractility differences were therefore expressed by the stimulation parameters. Since a longer PW and a shorter PER mean a better ability to contract, we formed the contractility ratio: the quotient of PW and PER. The higher PW/PER, the better the contractility. By means of this number, the ontogeny of uterine contractility in late pregnancy in the rat can be expressed numerically *in vitro.*

It is well known that sex hormones play an important role in the control of pregnant uterine contractility. A relative predominance of progesterone decreases the contractility, while a relative estrogen predominance increases it [20,21]. In a search for a physiological correlation, regression analysis was carried out between E/P and PW/PER. A very close correlation was found, which means that the *in vitro* contraction capability characterized by PW/PER is in keeping with the physiological regularity.

One of the possible reasons for the elevation in PW/PER is the increased number of gap junctions, which are thought to be deeply involved in the maturation process of the pregnant myometrium. Their role in the uterine contractility is to form low voltage-dependent cell-to-cell communications, heightening the electrical coupling between the smooth muscle cells, which coordinates the contractions of labour [22]. Moreover, the connexins, the genes coding gap junctions are very sensitive to changes in sexual hormone levels; some of them are very sensitive to shifts in E/P ratio [23], which is confirmation of the increased contractility ratio in parallel with E/P.

Another possible explanation of the elevated contractility ratio is the change in number of the voltage-dependent Ca^{+} channels during pregnancy. The mRNA levels for these channels are increased before term and preterm labour and prevented by progesterone treatment [24].

It is also known that E/P regulates the number of binding sites of adrenergic receptors in the pregnant myometrium [25]. It was earlier demonstrated that a high density of α_1-receptors caused an increased spontaneous uterine motility in the postpartum rat myometrium *in vivo* [26]. Our study revealed that the density of the α_1-receptor is constantly 25–50 times higher than the β-receptor density, and the changes in α_1/β are mainly consequences of the changes in α_1-receptor concentration. Moreover, the very close correlation between PW/PER and α_1/β furnishes further evidence of the increased contractility caused by the high density of α_1-adrenergic receptors in the pregnant rat uterus. The weaker correlation between E/P and α_1/β is in keeping with the physiological fact that a high E/P mostly increases the sensitivity of the α_1-receptors, in spite of the great change in their numbers, especially at the end of pregnancy [27,28]. This means that the increase in α_1/β-receptor density does not occur strictly in parallel with the increase in E/P, in contrast with the situation for PW/PER.

The decreases in the ability to contract and in the value of E/P or α_1/β on day 20 can be regarded as a plateau phase in the maturation process of the pregnant myometrium. At this stage of our research we have no clear explanation as to the physiological background of this phenomenon, clarification of which requires further investigations. However, our results do not contradict, but rather slightly modify the current picture of the ontogeny in the contractility of the pregnant rat uterus.

The evidence of our results leads us to suggest that EFS studies and PW/PER may allow an *in vitro* characterization of the ability of the pregnant rat uterus to contract. We further pre-

sume that this model can be suitable for the testing of the pharmacological effects of drugs influencing the uterine contractility.

The *in vivo* contraction curves of the rat myometrium near labour are rhythmic, without significant changes in their baseline tone [26]. Optimization of PW and leads to maximum contractions *in vitro* which are most closely similar to the physiological contractions observed during labour. Such regular maximum contractions can not be elicited by oxytocin or prostaglandins *in vitro. In vitro* pharmacological studies may therefore furnish more reliable information on such a model. It is suggested that pathophysiological conditions, eg. diabetes [29] or hypertension [30], and pharmacotherapy [31] may change the contractility response of the pregnant uterus. Our model may offer an experimental possibility to detect these changes by the relatively simple determination of the contractility ratio.

As far as we aware, this is the first *in vitro* model which provides a numerical measure of the ontogeny of the contractility of the uterus in late pregnancy. Our current data may also suggest the significance of the α_1-adrenergic receptors in pregnant uterine contractility, which might open new perspectives in pharmacological control.

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References

1. Fu X, Moeberg C, Backstrom T, Ulmsten U. Effects of estradiol and interaction with progesterone or oxytocin on term human myometrial contractile activity. Acta. Obstet. Scand. 1996; 75(1):13–8
2. Roberts JM, Riemer RK, Bottari SP, Wa YY, Goldfien A. Hormonal regulation myometrial adrenergic responses: the receptor and beyond. J. Dev. Physiol. 1989; 11(3): 125–34
3. Miyoshi H, Boyle MB, Mackay LB, Garfield RE. Voltage-clamp studies of gap junctions between uterine muscle cells during term and preterm labor. Biophys. J. 1996; 71(3): 1324–34
4. Csapo AI. Model experiments and clinical trials in the control of pregnancy and parturation. Am. J. Gynecol. 1963; 85:359–79
5. Elmer M, Alm P, Thorbert G. Electrical field stimulation of myometrial strips from non-pregnant and pregnant guinea-pigs. Acta. Physiol. Scand. 1980; 108: 209–13
6. Bulat R, Kannan MS, Garfield RE. Studies of the innervation of rabbit myometrium and cervix. Can. J. Pharmacol. 1989; 67: 837–44
7. Morizaki N, Morizaki J, Hayashi RH, Garfield RE., A functional and structural study of innervation of the human uterus. Am. J. Obstet. Gynecol. 1989; 160(1): 218–28
8. Csapo AI. Effects of progesterone, prostaglandin F2 alpha and its analogue ICI 81008 on the excitability and treshold of the uterus. Am. J. Obstet. Gynecol. 1976; 124(4): 367–78
9. Török I, Csapo AI. The effects of progesterone, prostaglandin F2 alpha and oxytocin on the calcium-activation of the uterus. Prostaglandins 1976; 12(2): 253–69
10. Schulman H. Am. The comparative actions of uterine inhibiting drugs. J. Obstet. Gynecol. 1978; 130(6): 684–88
11. Gáspár R, Márki A, Zupkó I, Falkay G. Evidence of non-synaptic regulation of postpartum uterine contractility in the rat. Life Sci. 1998; 62(12): 1119–24
12. Maltier JP, Legrand C. Characterization of α-adrenoceptors in myometrium of preparturient rats. Eu. J. Pharmacol. 1985; 117: 1–13

13. Sherwood DO, Fields PA. Corpus Luteum Peptides. In: Knobil E, Neill JD, editors. Encyclopedia of Reproduction. San Diego: Academic Press, 1998. vol. 1, p 724
14. Tomita T. Electrical Properties of Mammalian Smooth Muscle. In: Bülbring E, Brading AF, Jones AW, Tomita T, editors. Smooth muscle. London: Edward Arnold, 1970.pp. 197–243
15. Moustafa FA. Changes in cholinergic and noradrenergic nerves in the pregnant and postpartum uterus of the albino rat and guinea pig. Acta. Anat. Basel. 1988; 132(4): 310–16
16. Schliep HJ, Döring HJ, Classen HG, Dehnert HO. Muscle Contractions. Freiburg: Biomesstechnik-Verlag March GmbH. 1986. p 57.
17. Taggart MJ, Wray S. Contribution of sarcoplasmic reticular calcium to smooth muscle contractile activation: gestational dependence in isolated rat uterus. J. Physiol. (Lond). 1998; 511(Pt 1): 133–44
18. Martin C, Chapman KE, Thornton S, Ashley RH. Changes in the expression of myometrial ryanodine receptor mRNAs during human pregnancy. Biochim. Biophys. Acta. 1999; 1451(2–3): 343–52
19. Kawarabayashi T, Shojo H, Tsukamoto T. Effects of oxytocin, prostaglandin F2 alpha and prostaglandin E2 on intracellular free calcium concentrations of longitudinal muscle cells isolated from term pregnant rat myometrium. Gynecol. Obstet. Invest. 1997; 43(3): 145–49
20. Gitteus LN, Wojtczuk A, Weiss G, Goldsmith LT. Endothelin stimulation of rat uterine segment contractility is estrogen dependent. Gynecol. Obstet. Invest. 1998; 46(1): 49–53
21. Badia E, Nicolas Jc, Haiech J, Crastes De Paulet A. Effect of steroid hormones on the regulation of uterine contractility. Pflugers. Arch. 1986; 407(6): 670–676
22. Miyoshi H, Boyle MB, Mackay LB, Garfield RE. Voltage-clamp studies of gap-junctions between uterine muscle cells during term and preterm labor. Biophys. J. 1996; 71(3): 1324–34
23. Grummer R, Traub O, Winterhager E. Gap junction connexin genes cx26 and cx43 are differentially regulated by ovarian steroid hormones in rat endometrium. Endocrinology 1999; 140(6): 2509–16
24. Tezuka N, Ali M, Chwalisz K, Garfield RE. Changes in transcripts encoding calcium channel subunits of rat myometrium during pregnancy. Am. J. Physiol. 1995; 269(4 Pt 1): C1008–17
25. Williams LT, Lefkowitz RJ. Regulation of rabbit myometrial alpha adrenergic receptors by estrogen and progesterone. J. Clin. Invest. 1977; 60(4): 815–18
26. Zupkó I, Márki Á, Gáspár R, Falkay G. Correlation between α_1/β-adrenoceptors ratio and spontaneous uterine motor activity in the post-partum rat. Mol. Hum. Rep. 1998; 4(9): 921–24
27. Riemer RK, Goldfien, Roberts JM. Estrogen increases adrenergic- but not cholinergic-mediated production of inositol phosphates in rabbit uterus. Mol. Pharmacol. 1987; 32(5): 663–68
28. Riemer RK, Goldfien, Roberts JM. Rabbit myometrial adrenergic sensitivity is increased by estrogen but is independent of changes in alpha adrenoceptor concentration. J. Pharmacol. Exp. Ther. 1987; 240(1): 44–50
29. Reece EA, Sivan E, Francis G, Homko CJ. Pregnancy outcomes among women with and without diabetic microvascular disease (White's classes B to FR) versus non-diabetic controls. Am. J. Perinatol. 1998; 15(9): 549–55
30. Samadi AR, Mayberry RM. Maternal hypertension and spontaneous preterm births among black women. Obstet. Gynecol. 1998; 91(6): 899–904
31. Briggs GG. J. Medication use during the perinatal period. Am. Pharm. Assoc. (Wash). 1998; 38(6): 717–26