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Calorimetric investigation of rat myometrium as a function of gestational age

István Zupkó[a], Gábor Csík[a], George Falkay[a], Géza Regdon Jr[b], Dénes Lőrinczy[c,*]

[a] Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary
[b] Department of Pharmaceutical Technology, University of Szeged, Szeged, Hungary
[c] Biophysical Department, University of Pécs, Faculty of Medicine, H-7624 Pécs, Szigeti u. 12, Hungary

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ABSTRACT

The aim of the present work was the characterization of the myometrial samples of healthy control and chronic inflammation-exposed rats as a function of gestational stage by means of differential scanning calorimetry (DSC). Non-pregnant, mid-pregnant (day 14) and late-pregnant (day 21) animals were included. The spontaneous, KCl-stimulated as well as maximal motor activity was additionally recorded in vitro. Calorimetric enthalpy (ΔH) normalised on wet mass significantly increased as pregnancy progressed in case of non-inflamed uteri. Inflammation resulted in a significant decrease in $\Delta H/m$ of samples from non-pregnant till pregnant rats. Maximal contractility increased by inflammation in all tested stages of gestation. Our present results indicate that DSC can be considered as a useful tool for the investigation of the uterine function modified by gestation or a pathological condition.

1. Introduction

The uterus can be regarded as the maternal organ which undergoes the most dramatic change: pregnancy. Beside the well characterized several-fold increase in the mass and volume of the myometrial smooth muscle, a large body of evidence is available concerning the ultra structural development of the uterus during pregnancy [1]. All of these morphological changes are subordinated to the development of the fetus. The enlargement of the uterus is obviously required by fetal growth and the ultra structural remodelling seems to play an essential role in maintaining the quiescence of the myometrium during gestation.

The adrenergic system, besides prostaglandines and oxytocin, in the genitourinary tract is considered a crucial factor in the regulation of myometrial contractility, which is in line with the fact that currently available drugs used for the relaxation of the pregnant uterus are acting through β_2-adrenoceptors. α-Adrenoceptor antagonists have been suggested as alternative uterus relaxants with a more beneficial tolerability profile. Their efficacy is well characterized experimentally, but the clinical results are not yet available. Since inflammation has a modulating action on the adrenergic functions (e.g. adrenoceptor expressions), it seems conceivable that it can also affect the motor activity of the pregnant myometrium.

It is generally accepted that inflammatory procedures could be responsible for a substantial part of premature deliveries. The role of intrauterine infections in early contractions is confirmed by a large body of evidence [2]. Inflammations remote from the reproductive tract (e.g. periodontitis) have also been proposed as a causative factor of preterm labor, but the available clinical data are still limited [3].

Thermoanalytical techniques have been traditionally used for monitoring the physicochemical parameters of chemically pure substances (e.g. melting point and melting range, thermal stability, etc). Differential scanning calorimetry (DSC) can also be used for studying more complex systems such as biological samples (e.g. tissues or body fats) [4,5]. The few studies which have been published so far focus on the general examination of rat uterus or on the effect of ADP and ATP exerted on the uterus, and there are also some publications which investigate the thermal behaviour of non-pregnant and late pregnant human uterus [6,7]. Recently, we described a relationship between calorimetric enthalpy and the motor activity of the uterus of early pregnant rats [8].

The aim of the present study was the calorimetric investigation of uterine samples from healthy control and systemic inflammation-exposed non-pregnant, mid- and late-pregnant (day 14 and 21, respectively) rats. In order to characterize the possible relationship between calorimetric behaviour and physiological function, the spontaneous, KCl-stimulated and maximal motor activities of uterine rings from the same experimental design were additionally recorded.

* Corresponding author.
E-mail address: denes.lorinczy@aok.pte.hu (D. Lőrinczy).

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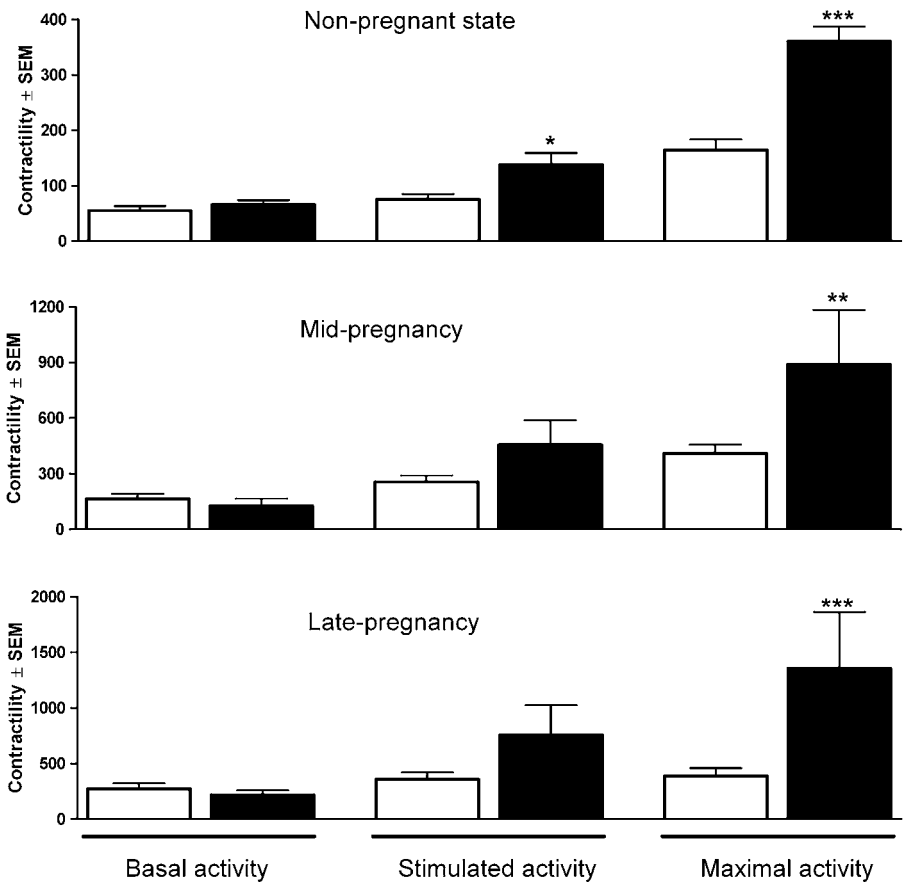

Fig. 1. Basal (spontaneous), stimulated (25 mM KCl) and maximal (70 mM KCl) motor activities of uterine rings as a function of gestational state. Uterine samples were excised for healthy control (□) and inflammation-exposed (■) rats. Columns mean the area of integrated tension in arbitrary units ±SEM ($n = 5$). *, ** and ***: $p < 0.05$, $p < 0.01$ and $p < 0.001$ as compared to the corresponding control values, respectively. Note the different scales in the three graphs.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats (200–250 g) were mated in a special cage in the early morning; copulation was determined by the presence of a copulation plug or sperm in a native vaginal smear. The day of conception was considered to be the first day of pregnancy. All experimental animal protocols satisfied the Guidelines for Animal Experimentation approved by the Animal Experimentation Committee of the University of Szeged.

Some of the involved animals were exposed to adjuvant arthritis (AA) as a tool to model generalized inflammation. AA in rats has been described by Pearson and Wood as exhibiting many similarities to human rheumatoid arthritis, and therefore it can be regarded as an animal model of the disorder [9]. Adjuvant was prepared by mixing killed *Mycobacterium butyricum* (Becton Dickinson, Budapest, Hungary) with paraffin oil to a final concentration of 5 mg/ml. AA was induced by a subplantar injection of adjuvant (0.1 ml) between the third and fourth digits of the right hind paw. Approximately 3–5 days are needed for the development of primary reaction (i.e. the inflammation in the injected hind paw) while the secondary reaction (i.e. generalized inflammation) is obvious after 10–12 days [10]. Pregnant animals were treated with *M. butyricum* on the 1st day of pregnancy and the experiments were performed on day 14 or 21. Non-pregnant rats were utilized at least 10 days after adjuvant injection.

2.2. Differential scanning calorimetry

During the calorimetric experiments the pieces of samples from control and AA-exposed non-pregnant, as well as 14 and 21 day pregnant uteri have been excised and kept at −70 °C until measurement. The thermal denaturation was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 °C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments. The sample and reference vessels were equilibrated with a precision of ±0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The data treatment after ASCII conversion was done by OriginPro 7.5. The calorimetric enthalpy change was determined with the aid of SETARAM two points fitting integration software. All of the results relating to DSC scans are the means ±SD from four measurements. Pregnancy-related differences were evaluated by one-way ANOVA, followed by Dunnett's multiple comparison test and inflammation-dependent changes were characterized by unpaired *t*-test (GraphPad Software, San Diego, CA, USA).

2.3. Isolated organ experiments

Dissected uterine rings from the same experimental design were vertically mounted in a tissue bath containing 10 ml de Jongh buffer. The composition of the buffer was 137 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 4 mM Na_2HPO_4 and 6 mM glu-

cose, at pH 7.4. Organ baths were kept at 37 °C and oxygenized. The integrated tensions of the rings were measured with a strain gauge transducer and recorded with Isosys Data Acquisition System (Experimetria Ltd., Budapest). The initial tension of the rings was set to 1.5 g and samples were equilibrated for 90 min before the experiment. After this period a 7-min period of spontaneous activity was recorded, and then stimulated contractions were elicited by 25 mM KCl. Finally, maximal tensions were determined by adding 70 mM KCl used for the normalization of the tensions. All of the presented results relating to tissue organ contractilities are the averages of the data from five independent experiments. The contractility data were evaluated by one-way ANOVA, followed by Newman–Keuls multiple comparison by means of GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

Preterm labour occurs in approximately 7% of all pregnancies, accounts for 75% of neonatal mortality and causes significant neonatal mortality. The exact reasons for early uterine contractions are poorly understood but at least one third of the cases are related to the infection or inflammation of the genital tract. As a substantial portion of these cases is subclinical, it is really difficult to estimate the role of these factors in premature labour [11]. On the other hand, inflammation is not necessarily harmful. A growing body of evidence suggests that physiological phenomena in reproduction, e.g. implantation, are closely related to inflammatory reaction [12].

The recorded motor activities of isolated uterine samples exhibited substantial gestational stage and inflammation dependent differences in contractility. Generally, as pregnancy progressed, both the spontaneous and stimulated activity of the myometrium increased. Inflammation resulted in a significantly elevated motor activity when the smooth muscle was stimulated with KCl (Fig. 1). Inflammation could significantly increase both stimulated (25 mM) and maximal contractility (elicited with 70 mM KCl) in non-pregnant rats. During pregnancy, on both investigated days, inflammation caused a similar contractility pattern but only the maximum contractility differed significantly.

It was found previously that experimental colitis and bladder inflammation decreased the spontaneous motor activity of the uterus as well as the uterotonic effect of oxytocin [13,14]. However, in these studies the uterine activity was recorded shortly after the induction of inflammation, therefore it could not be considered as a chronic condition. The present experiments were performed on animals exposed to adjuvant arthritis, which is generally regarded as an experimental model of human rheumatoid arthritis implicating that uterine functions were affected by a chronic condition.

Our DSC results indicate that inflammation in non-pregnant animals results in a slight but not significant increase in calorimetric enthalpy without change in T_m values. In non-pregnant rats, therefore, inflammation can alter ΔH values parallel with the maximal contractility elicited by a high concentration of KCl. On the other hand, more substantial and statistically significant inflammation related differences were detected when samples of pregnant animals were tested (Fig. 2, Table 1).

In control animals, as gestation progressed, transition enthalpy increased. The main calorimetric parameters exhibited inflammation-dependent differences in the tissue samples of pregnant animals. This difference is supported by the shapes of DSC curves too, which show appreciable divergences. Similar results were reported recently from an investigation of human myometrial samples [7]. These gestation-related differences may be explained by a gradual increase in the tension on the uterine wall, meaning a more organized structure of the smooth muscle.

The exact characterization of the link between the ultrastructure of the developing pregnant uterus and its calorimetric behaviour is

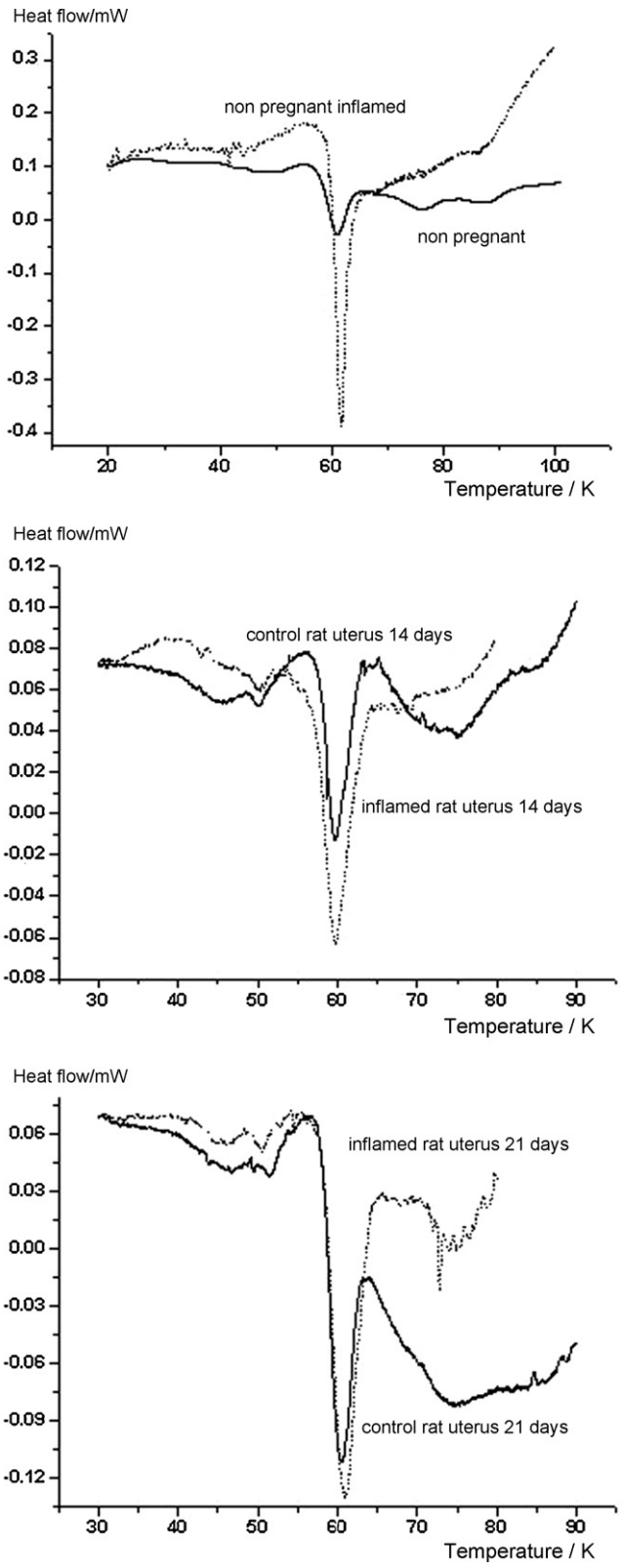

Fig. 2. Representative DSC scans of uterine samples from non-pregnant, 14 and 21-day pregnant rats. The solid lines stand for control, the short-dash lines for the inflammation-exposed animals, respectively.

Table 1
The melting temperatures and calorimetric enthalpy changes normalised for wet mass of control and inflammation-exposed uterine samples as a function of gestational state. Data are means ± SD ($n = 4$). $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ as compared with the non-pregnant values, respectively. $^{*}p < 0.05$ and $^{***}p < 0.001$ as compared with the control conditions, respectively.

	Non-pregnant	Inflamed non-pregnant
T_m (°C)	61.0 ± 0.26	61.07 ± 0.41
ΔH (J g^{-1})	2.43 ± 0.27	2.85 ± 0.28
	14 days control	14 days inflamed
T_m (°C)	59.17 ± 0.26$^{\#\#}$	59.93 ± 0.37$^{\#\#,*}$
ΔH (J g^{-1})	2.66 ± 0.26	2.13 ± 0.22$^{\#\#,*}$
	21 days control	21 days inflamed
T_m (°C)	60.44 ± 0.27$^{\#}$	60.35 ± 0.31$^{\#}$
ΔH (J g^{-1})	3.54 ± 0.25$^{\#\#}$	2.36 ± 0.24$^{\#,***}$

beyond the aim of the present study. A wide variety of components of uterine muscle, including receptors, transmitters and growth factors, changes characteristically during gestation but none of them could be indicated as an explanation of higher transition enthalpy. Gap junctions are considered to participate in the regulation of myometrial motor activity. The frequency of these cellular connections changes too during pregnancy and their presence prior to or during labor has important physiologic implications in delivery [15]. Gap junction is a specialized intercellular connection between animal cells. Since it directly connects the cytoplasm of two cells which allows various molecules and ions to pass freely between cells it is considered to be the route by which the excitation spreads within the myometrium. Therefore, the higher expression of gap junctions in late pregnancy makes the myometrium more coherent and its role in the altered thermal character can be suggested.

Non-pregnant intact uteri were exposed to two crucial stimuli: pregnancy and serious generalized inflammation. Since inflammation affected the myometrial calorimetric values relatively slightly in samples with pregnancy, it seems conceivable that pregnancy itself initiates a kind of "resistance" against inflammation. It is in line with a large body of clinical evidence indicating that physiological pregnancy exerts a beneficial effect on chronic inflammatory disorders [16,17]. As an alternative explanation it could be suggested that the pathological condition is not able to "overwrite" the ultra-structural changes induced by gestation, which protection is evidenced by our present calorimetric results. A previous superfusion study stated that experimental diabetes induced the most pronounced deterioration in the adrenergic nerve functions of non-pregnant rats while its effect was insignificant in late-pregnancy [18]. As a summary, our results imply that thermal analysis could be applied to characterize the pathological conditions of animal tissues.

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References

[1] E.B. Haase, J. Buchman, A.E. Tietz, L.P. Schramm, Cell Tissue Res. 288 (1997) 293.
[2] M.A. Elovitz, Semin. Fetal Neonatal Med. 11 (2006) 327.
[3] M. Klebanoff, K. Searle, BJOG 113 (Suppl 3) (2006) 43.
[4] G. Sohár, E. Pallagi, P. Szabó-Révész, K. Tóth, J. Therm. Anal. Calorim. 89 (2007) 853.
[5] P. Than, D. Lőrinczy, Thermochim. Acta 404 (2003) 149.
[6] D. Lőrinczy, Z. Vértes, J. Belágyi, Thermochim. Acta 376 (2001) 109.
[7] F. Wilhelm, K.A. Kovács, Z. Vértes, D. Lőrinczy, J. Therm. Anal. Calorim. 89 (2007) 863.
[8] G. Csík, I. Zupkó, G. Regdon, G. Falkay, D. Lőrinczy, J. Therm. Anal. Calorim. 95 (2009) 695.
[9] C.M. Pearson, F.D. Wood, Arthritis Rheum. 2 (1959) 440.
[10] H.G. Vogel (Ed.), Drug Discovery and Evaluation. Pharmacological Assays, Springer Verlag, Berlin, 2002.
[11] R. Romero, J. Espinoza, M. Mazor, Fertil. Steril. 82 (2004) 799.
[12] V. Blank, E. Hirsch, J.R. Challis, R. Romero, S.J. Lye, Placenta 29 (Suppl A) (2008) S102.
[13] N. Dmitrieva, O.L. Johnson, K.J. Berkley, Neurosci. Lett. 313 (2001) 49.
[14] E. Houdeau, M. Larauche, R. Monnerie, L. Bueno, J. Fioramonti, Am. J. Physiol. Regul. Integr. Comp. Physiol. 288 (2005) R630.
[15] R.E. Garfield, S. Rabideau, J.R. Challis, E.E. Daniel, Am. J. Obstet. Gynecol. 133 (1979) 308.
[16] R.H. Straub, F. Buttgereit, M. Cutolo, Ann. Rheum. Dis. 64 (2005) 801.
[17] V.R. Tandon, S. Sharma, A. Mahajan, V. Khajuria, A. Kumar, Indian J. Med. Sci. 60 (2006) 334.
[18] G. Falkay, G. Spiegl, D. Csonka, I. Zupkó, Neurochem. Int. 51 (2007) 306.