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DSC INVESTIGATION OF EARLY PREGNANT UTERUS OF THE RAT

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The aim of the present work was the characterization of nonpregnant and early pregnant myometrium (days 3–6) of the rat by means of differential scanning calorimetry (DSC). The spontaneous motor activity as well as the KCl-evoked contractions of isolated uterine rings was additionally recorded. A relatively close correlation was found between calorimetric enthalpy (ΔH) and the contractility of the uterus samples. Our results indicate that DSC is a useful tool for the investigation of the functions of developing myometrium and it can be considered as supplementing the traditional structural and functional methods.

Keywords: *contractility, DSC, gestation, rat, uterus*

Introduction

Gestation induces plenty of dramatic changes in practically all the physiological functions in mammals, including hormonal environment, metabolic state, but the most impressive changes are restricted to the reproductive tract, especially to the uterus. The several-fold increase in the mass and volume of the myometrial smooth muscle is well-known and a large body of evidence is available concerning the ultrastructural development of the uterus during pregnancy [1]. These morphological changes include the gestation-dependent regulation of gap-junctions as well as the pregnancy-induced degeneration of nerve fibres supplying both the vasculature and the smooth muscle itself [2]. The structural transformations can be considered as the basis of consequential changes in the physiological parameters of myometrial contractility as well as its pharmacological behaviour [3]. It is presumed that all these features have a role in the maintenance of a continuous relaxed state in the course of pregnancy. Moreover, this uterine remodelling could be required not only for the quiescence during gestation, but also for the initation of contractions before delivery. Therefore, the thorough understanding of the pregnancy-induced structural-functional changes seems to be a precondition for the prevention of premature contractility.

Thermoanalytical techniques can be used for monitoring several important physicochemical phenomena such as e.g. determination of melting point and melting range, examination of polymorphic modifications, control of thermal stability, compatibility studies etc. It is suitable for examining not only monocomponent systems, active agents and auxiliary materials but also multiple systems e.g. multiple emulsions, ointments, pellets, tablets and other dosage forms as well as for studying materials and circumstances influencing the procedures of medicine production (e.g. film coating) [4–7].

DSC examinations can also be used for studying more complex systems such as biological samples (e.g. articular cartilages, bones and smooth muscle tissues, uterus, etc.) [8, 9]. The few studies which have been published so far focus on the general examination of rat uterus or on the effect of ADP, ATP exerted on the uterus, and there are also some publications which investigate the thermal behaviour of nonpregnant and late pregnant human uterus [10, 11]. However, none of the publications to date has performed such examinations about the early period of pregnancy, therefore the aim of our research was to examine samples of early pregnant uterus as a function of gestational age. Additionally, spontaneous and KCl-evoked motor activities of isolated uterine rings were recorded in order to find a relationship between the thermoanalytical behaviour and the contractility of the myometrium.

Experimental

Materials and methods

Animals

Sprague–Dawley rats (200–250 g for females) were mated in a special cage early in the morning and copulation was determined by the presence of sperm in a

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native vaginal smear. The day of conception was considered to be the first day of pregnancy. All experimental protocols satisfied the Guidelines for Animal Experimentation approved by the Animal Experimentation Committee of the University of Szeged.

Differential scanning calorimetry

During the calorimetric experiments the pieces of samples from nonpregnant, as well as 3-, 4-, 5- and 6-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^{-1}. 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 Origin 6.0. The calorimetric enthalpy change was determined with the aid of Setaram two points fitting integration software.

Isolated organ experiments

Dissected uterine rings from the same animals of gestational age 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 glucose, 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, Hungary). 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 then contractions were elicited by 25 mM KCl. Finally, maximal tensions were determined by adding 70 mM KCl used for the normalization of the tensions.

Results and discussion

Our results show that the calorimetric behaviour of the uterus samples basically differs as a function of gestational age (Fig. 1). In case of nonpregnant uterus the main transition temperature (T_m) was 61°C with 0.61 J g^{-1} calorimetric enthalpy (ΔH) change. During the development of pregnancy these parameters altered to 61.25°C and 1.18 J g^{-1} (3^{rd} day); 60.7°C and 0.83 J g^{-1} (4^{th} day); 60.6°C and 2.05 J g^{-1} (5^{th} day); 60.4°C and 1.21 J g^{-1} (6^{th} day).

The contractility of a uterine ring was expressed in percent as the integrated tension belonging to 7 min and the maximal contracture, elicited by a high concentration of KCl, was considered as 100%. Beside(s) the basal activity, a low concentration (25 mM) was used to test the excitability of the muscle. It was found that uterine rings from the 5^{th} day of pregnancy exhibited a significantly higher motor activity, both spontaneous and K-stimulated (Fig. 2). A relatively

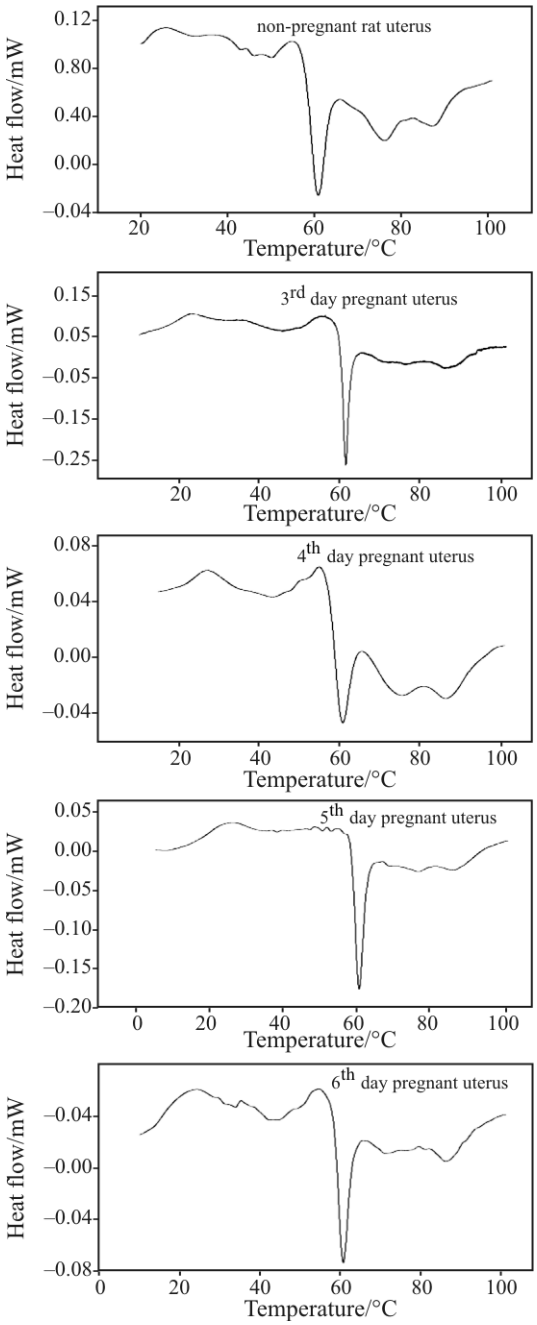

Fig. 1 DSC scans of non-pregnant and early pregnant (days 3, 4, 5 and 6) rat uterus

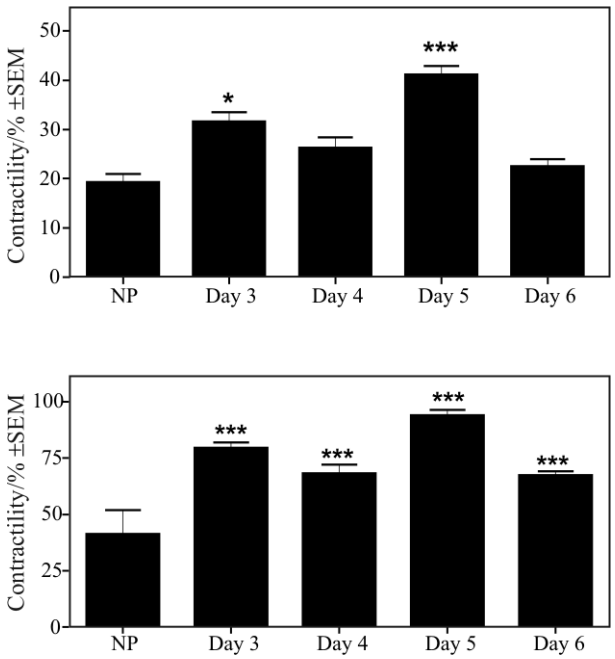

Fig. 2 Spontaneous (upper panel) and 25 mM KCl-stimulated (lower panel) motor activities of non-pregnant (NP) and early pregnant uteri. * and *** denote $p<0.05$ and $p<0.001$ when compared with the non-pregnant values, respectively

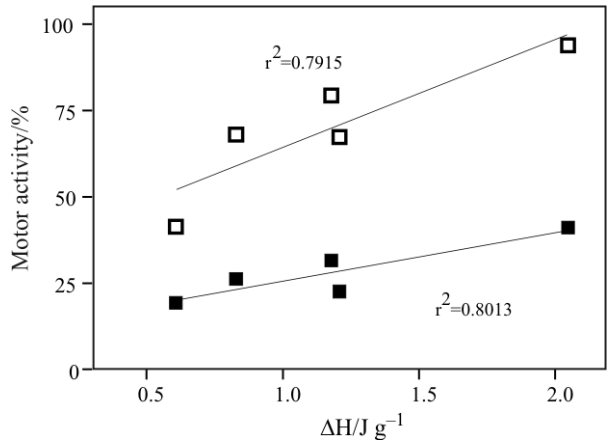

Fig. 3 Correlation of calorimetric enthalpy and motor activity of the non-pregnant and early pregnant uterus of the rat. ■ and □ denote spontaneous and 25 mM KCl-stimulated motor activities, respectively

close correlation was revealed between calorimetric enthalpy (ΔH) and both basal and stimulated contractility (Fig. 3).

Conclusions

The aim of the present work was the characterization of non-pregnant and early pregnant (days 3–6) myometrium of the rat by means of DSC. The basal contractile activity as well as the KCl-evoked contractions of isolated uterine rings was additionally recorded in order to find a relationship between the parameters describing calorimetric behaviour and contractility. We found that the early pregnant myometrium was generally more active than the non-pregnant one, and both basal and KCl-stimulated activity exhibited its maximum on day 5. The reason for this feature is not completely understood but as that is the day of implantation in the 22-day gestation in the rat, it is speculated that increased contractility is required for the transfer of the zygotes to the sites of implantations [12]. The correlation found between the motor activity and the calorimetric character (ΔH) implicates that contractility can be determined or approximated by DSC. Most of the functional smooth muscle methods require freshly prepared tissue samples (i.e. isolated organ experiments). DSC however, can be performed with tissue samples kept at –70°C between excision and measurement, offering the possibility of measurement independently of the in vivo phase.

Our current knowledge about the regulation of the motor activity of the pregnant myometrium is incomplete and the scenario of the initiation of labour is also enigmatic. The exact reason for premature uterine contractions is not elucidated either, but it leads to premature delivery affecting approximately 10% of all births, which account for 75% of neonatal mortality [13, 14]. The currently available uterus relaxing agents, acting as agonists on β2-adrenergic receptors, can stop premature contractions but the acute efficacy of these drugs does not mean a breakthrough in the obstetrical practice. Beside the serious side effect that fast and frequent tachyphylaxis also limits the therapeutic value of these tocolytics. All of these facts clearly demonstrate the importance of investigations targeting the physiology of the pregnant myometrium as well as the possibilities for pharmacological intervention. Our present results indicate that DSC can be considered as a reliable tool for the functional investigation of the developing myometrium.

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