Structural Transformations of Myocytes during Gestation and Early Postpartum Involution of the Uterus

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Myocytes with large homogeneous vacuoles and numerous protrusions of the cytoplasm into extracellular space (manifestations of clasmatosis) were detected in rat myometrium during normal gestation and early postpartum period. The percentage of vacuolated smooth-muscle cells and numerical density of inflammatory cells in the myometrium were evaluated. The data indicate the absence of typical inflammatory reaction in the myometrium during elimination of smooth-muscle cells in the course of postpartum involution. Clasmatosis and apoptosis are hypothesized to be the main mechanisms providing reduction of the uterine weight after delivery.

Key Words: *uterus; involution; myocytes; clasmatosis; apoptosis*

The weight of the uterus increases significantly during gestation and very rapidly returns to the initial (before pregnancy) level after delivery. For example, in rats the weight of the uterus increases almost by one order of magnitude [12], while after delivery is returns to the initial level within 5 days. So intense transformations indicate potent adaptive potentialities of the uterine tissues and appreciable velocity of the biological processes providing these changes under physiological conditions. The weight of the uterine myometrium increases at the expense of the size and number of smooth-muscle cells (SMC) [4], the contribution of each of these processes being about the same. Postpartum involution of the myometrium is presumably realized through the following intracellular mechanisms: myocyte clasmatosis leading to cell shrinkage [7], autophagocytosis (occurring mainly in the fibroblasts) [6], and apoptosis surely eventuating in cell elimination [8]. Autophagocytosis usually manifests in fibroblasts; procollagen molecules, synthesized, but not yet assembled in a mature chain, are thus destroyed. This process involves the myocytes to a lesser degree and, speaking about these cells, we can neglect it. Apoptosis can be registered by histological methods only during its final stage (by apoptotic bodies containing the nuclei in a state of pyknosis, karyorrhexis, and karyolysis). Myocyte clasmatosis is detected at the light microscopic level, and hence, its intensity can be evaluated directly by the percentage of SMC involved in this process. The possible role of necrobiosis processes in the organ weight reduction can be evaluated by the presence of cells in this state and signs of inflammation (as the so-called inflammation cells and infiltrate from them).

We carried out a morphometrical study of clasmatosis and apoptosis of uterine SMC during pregnancy and early postpartum involution and evaluated the probability of an inflammatory process development as a result of myocyte death.

MATERIALS AND METHODS

The study was carried out on 55 adult female Wistar rats (160-180 g). The uteri were examined on

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days 10 and 21 of pregnancy and during the first 5 days postpartum, when fulminant processes of uterine involution develop. The uteri of nonpregnant animals served as the control. Pregnancy term was determined starting from the day of spermatozoa detection in vaginal smears.

The animals were sacrificed by decapitation under ether narcosis. The placental zone of the uterus (participating in the formation of the placenta) and the interplacental zone located between the placentas of the neighboring fetuses were collected for the analysis. Material for histological study was fixed in 10% aqueous solution of formalin, dehydrated in ascending alcohols, and embedded in paraffin [1]. The sections (5-7 μ) were stained by the method of van Gieson. Specimens for electron microscopy were fixed in 1% OsO_4 in phosphate buffer (pH 7.4-7.6), dehydrated in alcohols, and embedded in epon. Ultrathin sections were contrasted with aqueous solutions of uranyl acetate and lead citrate [3] and examined under a transmission electron microscope at ×4000.

Structural changes in the myometrium during pregnancy and involution were evaluated by morphometry.

The percentage of vacuolated myocytes (VM) was evaluated in histological sections at the final magnification of 630. A total of 50-70 visual fields (65-120 cells each) were analyzed for each period of observation. The numerical density (ND) of cells participating in inflammatory reaction (macrophages, neutrophils, lymphocytes) and of apoptotic bodies was evaluated in a visual field at the same magnification and estimated per μ^2 of myometrial section.

All numerical data were statistically processed using Student's *t* test; the differences between the means were considered significant at $p<0.05$ [2].

Changes in all morphometrical parameters were largely similar in all the studied areas of the uterus (placental and interplacental), and we discuss the data on the myometrium without specifying the area of the organ.

RESULTS

The myometrium of nonpregnant animals contains very few SMC with vacuoles. On day 10 of pregnancy, vacuole-like formations with homogeneous contents of low electron density were detected in the entire cytoplasm volume of many myocytes. Later (day 21 of pregnancy) numerous formation of this type were detected also in the extracellular space. These cells with vacuoles and cytoplasm protrusions were easily discernible under a light microscope.

According to morphometric analysis, the percentage of VM in the myometrium on day 10 of pregnancy was already significantly ($p<0.001$) higher than in control animals, and on day 21 (before delivery) the number of VM increased still more (Fig. 1). During the first 4 days of postpartum involution of the uterus, the percentage of VM varied from 10 to 20, and on day 5, their percentage in the SMC population remained high.

The formation of vacuoles in SMC and protrusion of the cytoplasm sites (clasmatosis) in uterine myocytes were described previously [7]. Presumably, cytoplasm protrusions with subsequent separation are a reaction of hypertrophic myocytes to possible hypoxia because of uterine vessels stenosis after labor. Presumably, this mechanism provides urgent reduction of cell size as adaptation under conditions of resources shortage. High percentage of VM starting from day 10 of normal pregnancy (Fig. 1) can be due to poor oxygen supply to the myometrium before labor.

The results of electron microscopy and morphometric analysis indicate that the mechanisms of cell elimination in the myometrium by clasmatosis and apoptosis are triggered long before labor. However, it is obvious that plastic processes predominating in the uterus lead to its enlargement. Directly after delivery, the level of anabolic processes decreases, while clasmatosis mechanisms, which started developing during pregnancy, lead to a rapid shrinkage of myocytes. Hence, the greater part of VM in rat myometrium on day 5 postpartum corresponds to active progress of involution. Therefore, recovery of the uterine weight on day 5 postpartum [12] does not mean that the involution processes are over in rats by this period.

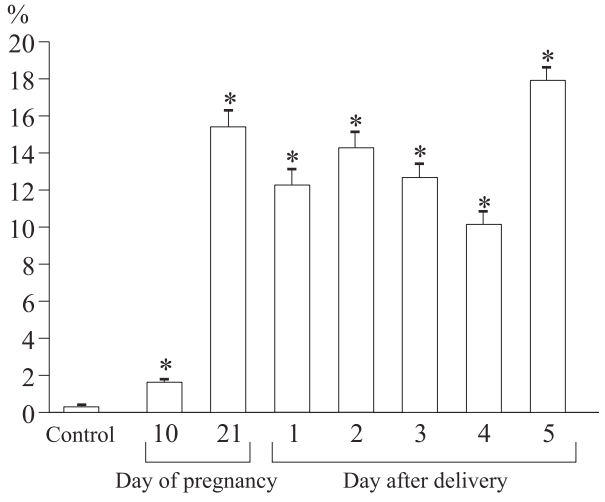

Fig. 1. Percentage of VM in the rat myometrium during pregnancy and postpartum involution. *$p<0.001$ compared to the control.

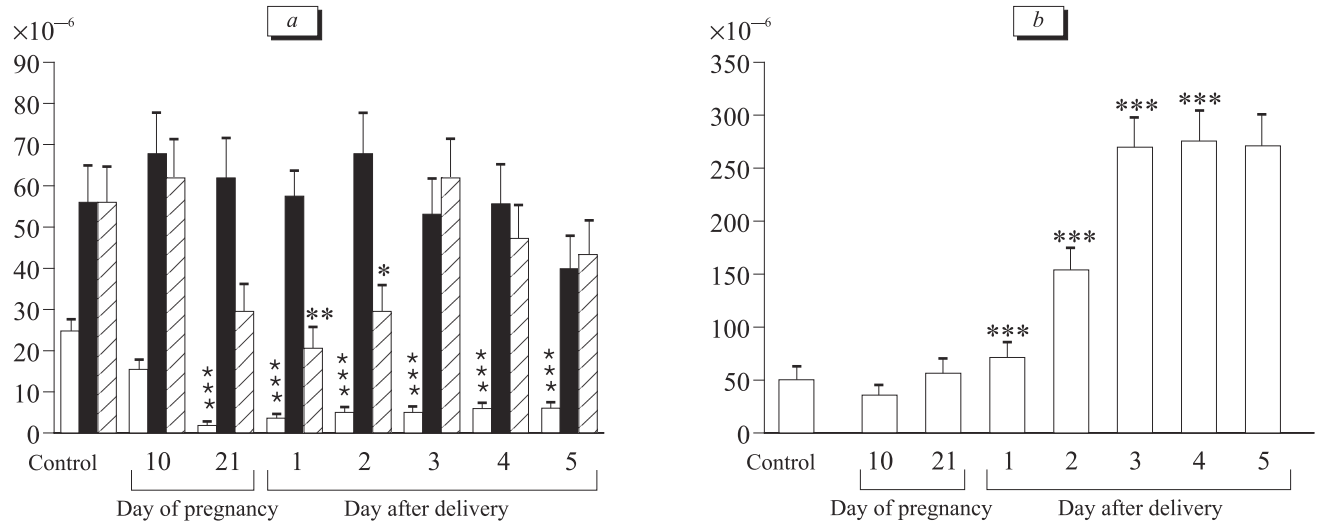

Fig. 2. Numerical density of hematogenic cells (*a*) and apoptotic bodies (*b*) per μ² of the rat myometrial section during pregnancy and postpartum period. Light bars: neutrophils (*10); dark bars: macrophages; cross-hatched bars: lymphocytes. *p<0.05, **p<0.01, ***p<0.001 compared to the control.

The study of the time course of numerical density (ND) of hematogenic ("inflammation") cells did not confirm the development of inflammatory processes in the uterus [9]. The lymphocyte ND decreased (p<0.01) during pregnancy and returned to the control level on day 3 postpartum (Fig. 2, *a*). A similar regularity was observed during pregnancy for neutrophils, but their ND remained low after labor (Fig. 2, *a*). Presumably, one of the key mechanisms providing the restructuring of the myometrium during this period is (in addition to clasmatosis) apoptosis, also inducing no inflammation processes. ND of cells containing fragments of nuclei (one of the final stages of apoptosis) increased in the myometrium after delivery, and its high level persisted until the end of involution (Fig. 2, *b*). Elimination of a great number of myocytes by this mechanism did not lead to inflammatory reaction, which was seen from the absence of the increase in the hematogenic cell ND. Uterocalin (protein; specific inductor of apoptosis in neutrophils [10], not affecting macrophages [5]) accumulates in uterine tissues before labor. Our results showed no structural manifestation of inflammation effects, providing intense inflow of macrophages. ND of macrophages was constant during al periods of the study and corresponded to the value in nonpregnant animals (Fig. 2, *a*). Electron microscopy of the myometrium detected macrophages as cells of geometrically irregular shape with cytoplasmic protrusions containing well-expressed lysosomal system. Apoptotic bodies were often detected in secondary lysosomes.

Analysis of the results suggests the presence of organ-specific mechanisms in the postpartum myometrium, which limit the counts of neutrophils without affecting macrophages. This specific regulation of inflammation can be explained by the specific hormonal status during pregnancy and lactation. Elimination of myocytes with signs of clasmatosis is slow, and they form numerous cell conglomerations in the interstitium. Intact membranes in cells within these structures prevent the development of typical inflammation. Presumably, these conglomerations and vacuoles formed by the clasmatosis mechanism are used as plastic material in the form of monomers resultant from lysosomal hydrolysis in macrophages [11].

Hence, the weight of the uterus rapidly returns to the level before pregnancy during the early postpartum period due to reduction of myocyte size by the clasmatosis mechanism (separation of peripheral fragments of the cytoplasm), on the one hand, and to overall elimination of myocytes by apoptosis, on the other.

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