Elastase Involvement in Extracellular and Intracellular Collagen Degradation during Postpartum Involution of the Uterus

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The location of elastase in rat uterus during its postpartum involution was studied by electron histochemical method. Intense extracellular activity of the enzyme was detected: the reaction product was found on the cytolemma of smooth-muscle cells and, to a lesser extent, on the cytolemma of macrophages and fibroblasts and on adjacent collagen fibrils. The reaction product was also found in vacuoles with collagen in macrophages and fibroblasts.

Key Words: *postpartum involution of the uterus; collagen degradation; elastase; electron histochemistry*

The uterus during postpartum involution is considered to be the optimal model for studying collagenolytic processes. The amount of collagen in the uterus increases 4-5-fold during gestation. During postpartum involution collagen resorption in the uterus is extremely intense; in rats collagen content returns to normal within 4 days. The mechanisms of this rapid resorption are unknown. It was hypothesized that serine proteinases, *e.g.* elastase, play an important role in collagen degradation [1,4]. This lysosomal enzyme can cleave the main components of extracellular matrix *in vitro*: collagen, elastin, proteoglycanes, and glycoproteins [2,3,7,8]. Recent experiments showed that elastase activates procollagenase [5,9].

Despite numerous studies of elastase, its participation in collagen degradation *in vivo* was not proven.

We studied the location of elastase activity in the uterus during its postpartum involution by electron histochemical method.

MATERIALS AND METHODS

Rat uterus was examined 2 and 3 days postpartum, when elastase activity sharply increased. The animals were decapitated under ether narcosis. The material was histochemically treated for detecting elastase activity at an ultrastructural level by a previously described method [6] using Glutaryl-Ala-Ala-Ala-4MβNA (Bachem) for substrate. Control samples were incubated in a substrate-free medium. Ultrathin sections were not contrasted.

RESULTS

Examination of the myometrium showed thick collagen strata between smooth muscle cells (SMC) (Fig. 1, *a*). Elastase reaction product was detected in SMC lysosomes. Intense reaction was observed in the extracellular space. Appreciable amount of the reaction product in the form of individual granules or more or less homogeneous conglomeration of granules was seen on SMC cytolemma and directly on collagen fibrils adjacent to SMC and in fibrous strata (Fig. 1, *a*). This attests to the release of elastase into extracellular space by these cells.

In macrophages elastase reaction product was found in lysosomes and various vacuoles. Different numbers of vacuoles with collagen fibrils were seen in the cytoplasm of the majority of macrophages (Fig. 1, *b*). Granules of elastase reaction product were found in many vacuoles with collagen, indicating the phagocytic na-

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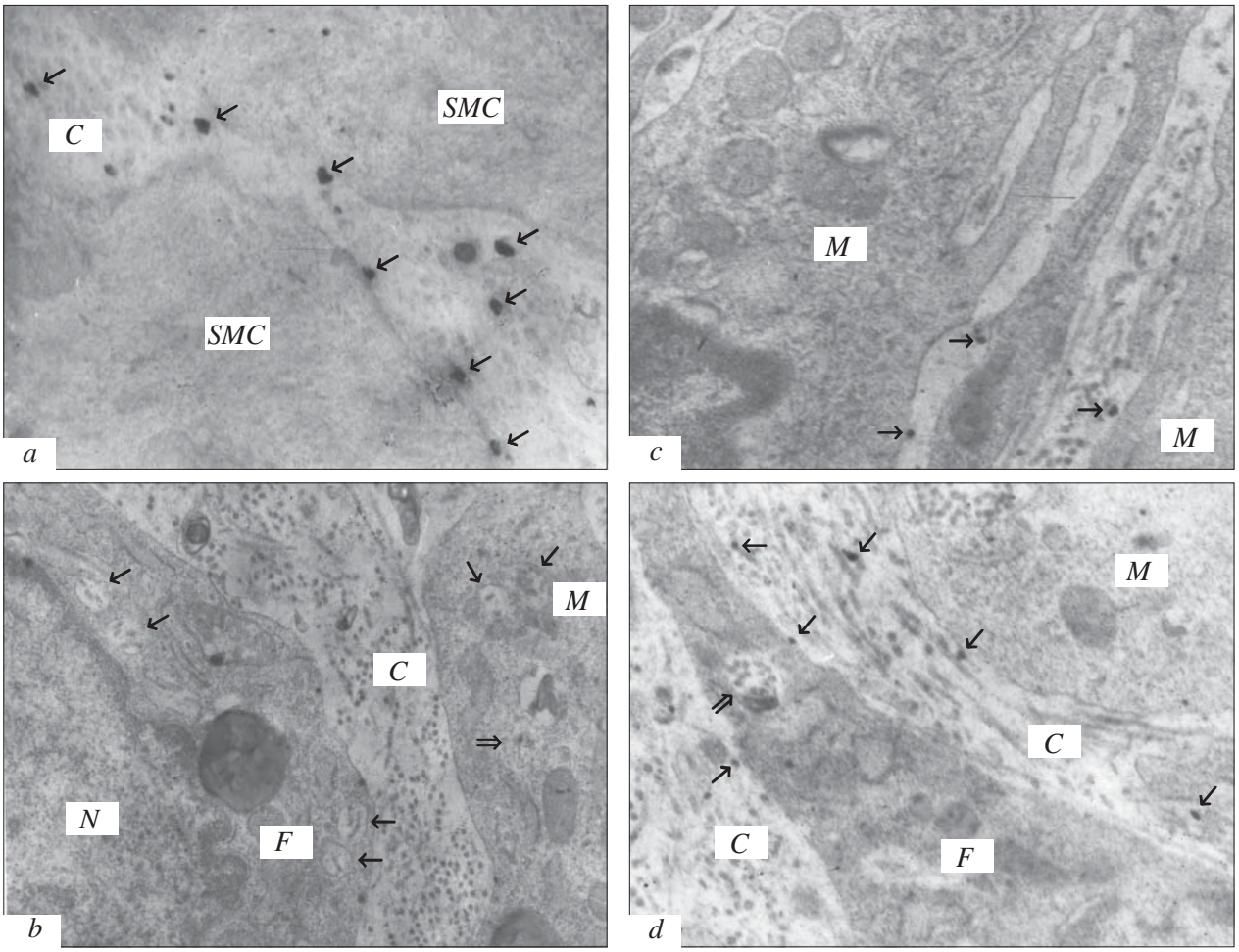

Fig. 1. Location of elastase in the uterus during its postpartum involution. *a*) reaction product (shown with an arrow) on day 3 postpartum is situated outside the cell on the smooth-muscle cell (*SMC*) cytolemma and on collagen fibrils (*C*), ×25,000; *b*) vacuoles with collagen (shown with an arrow) in the cytoplasm of a macrophage (*M*) and fibroblast (*F*) on day 3 postpartum. One vacuole contains the reaction product (shown with two arrows). *C*: collagen; *N*: nucleus, ×15,000; *c*) reaction product granules (shown with an arrow) on day 2 postpartum are situated outside the cell on the macrophage (*M*) cytolemmas, ×20,000; *d*) vacuole with collagen containing reaction product (shown with two arrows) in fibroblast (*F*) cytoplasm on day 2 postpartum. Reaction product granules (shown with an arrow) are seen on cytolemmas of fibroblast and macrophage (*M*) and on adjacent collagen (*C*) fibrils, ×20,000.

ture of these vacuoles (Fig. 1, *b*). Extracellular elastase activity was seen as the reaction product (most often in small amounts) in the form of individual granules on the macrophage cytolemma and on the adjacent collagen fibrils (Fig. 1, *c*).

Like in macrophages, vacuoles and phagolysosomes containing phagocytosed fragments of fibrils at different stages of degradation were found in the cytoplasm of many fibroblasts on postpartum days 2 and 3 (Fig. 1, *b*, *d*). Many phagolysosomes containing collagen contained also the granular reaction product, the marker of elastase activity (Fig. 1, *d*). In addition, we detected extracellular activity of the enzyme: few gra-nules were situated on the fibroblast cytolemma and on the adjacent collagen fibrils (Fig. 1, *d*). Control preparations contained no reaction product.

These data indicate that elastase is involved in intracellular resorption of collagen by macrophages and fibroblasts during postpartum involution of the uterus and is released mainly by SMC and less so by macrophages and fibroblasts into the extracellular space, where it actively participates in extracellular collagen degradation.

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