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Adrenomedullin Expression in the Human Endometrium

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Immunohistochemical studies were performed using a specific antibody to human adrenomedullin (AM) to determine its presence and cellular localization in the human endometrium, in the different phases of the menstrual cycle, and in the postmenopausal period. Specimens were obtained from 21 patients who underwent abdominal hysterectomy for various reasons. The endometrium had no pathological lesion in all cases. In the early and mid proliferative phases of the menstrual cycle, no immunostaining for AM was noted in the endometrium. AM immunostaining in the endometrium became apparent in the late proliferative phase. The staining intensity of AM in the endometrium became more abundant in the secretory phase. No appreciable difference in the staining intensity of AM in the endometrium was noted among early, mid, and late secretory phases. Immunostaining for AM was evident in both the epithelial and stromal compartments of the endometrium. In the postmenopausal endometrium, there was intense immunostaining for AM only in the stromal compartment. This is the first study to demonstrate the expression of AM in the endometrium in relation to the menstrual cycle. The results obtained suggest the participation of AM in the growth and differentiation of the endometrium.

Key Words: Endometrium; adrenomedullin; menstrual phase; postmenopausal stage.

Introduction

Adrenomedullin (AM) is a potent hypotensive peptide discovered from human pheochromocytoma tissue by its stimulating activity of platelet cyclic adenosine monophosphate (cAMP) production *(1,2)*. Subsequently, a cDNA clone encoding human AM precursor was isolated, and its nucleotide sequence was determined *(3)*. From the AM precursor, proadrenomedullin N-terminal 20 peptide was identified, characterized, and analyzed *(4–6)*.

The effects of this peptide on the cardiovascular system have been well documented *(7,8)*. AM is known to be secreted by endothelial cells *(9)* and to act directly on vascular smooth muscle cells, causing an increment on intracellular cAMP leading to relaxation *(10)*. AM has a natriuretic and diuretic effect *(11)*. AM causes dilatation of the vascular bed *(12,13)*, as well as bronchodilatation *(14)* and exerts possible protective action against pathogens *(15)*. This peptide has been described as an antisecretagogue in different systems *(15–18)*. AM also acts as a neuromodulator and a neurotransmitter in the brain and as a neurohormone in the pituitary *(19)*. Furthermore, Miller et al. *(20)* demonstrated a potential role of AM as an autocrine growth factor in human tumor cell lines.

Previous reports on the expression of AM in the female reproductive tract and fetal membranes in pregnancy suggest that this peptide may play an important role in the physiology of reproduction *(21–23)*. Available data on the expression of AM in the human endometrium describe no reference to the menstrual phase *(24,25)*. In the present study, cytological localization of AM in the endometrial glands and stromal components of the normal human endometrium was determined over the course of the menstrual cycle and also in the postmenopausal stage.

Results

The early proliferative phase is characterized by low columnar epithelial lining of glands having a simple tubular appearance. The stroma is dense in this phase. There is evidence of mitotic activity both in the glands and in the stroma. There was no AM staining in the epithelium of the endometrial glands and endometrial stroma in this phase (Fig. 1A). In the mid proliferative phase, elongation of glands is noticeable with the development of stromal edema. The glands are evidently tortuous in some areas. Although mitotic activity is present in both the epithelial and stromal compartments, there was no immunostaining detectable for AM in the mid proliferative phase (Fig. 1B). The late proliferative phase is characterized by increased tortuosity of the glands with subsiding stromal edema. Pseudostratification of columnar epithelium of the glands is evident. It is at this phase where mitotic activity is maximal and changes take place rapidly in the glands, stroma,

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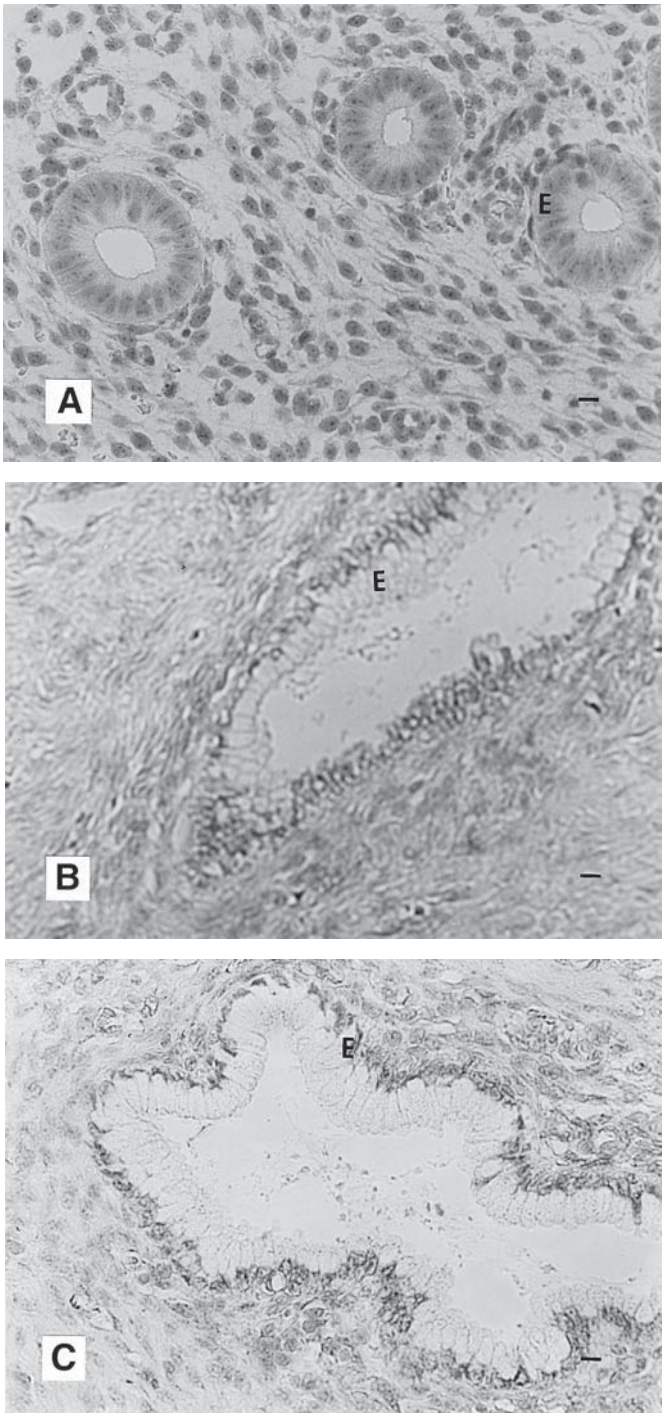

Fig. 1. Immunohistochemical localization of AM in the **(A)** early, **(B)** mid, and **(C)** late proliferative phases of the endometrium. There was no evident immunostaining for AM in the endometrial glands and endometrial stroma in both the early and mid proliferative phases. Weak immunostaining for AM was noted both in the epithelium of the endometrial glands and in the endometrial stroma in the late proliferative phase. E, epithelium of the endometrial gland; S, endometrial stroma. Bars represent 10 μm. Original magnification: ×400.

and blood vessels. This late proliferative phase was the earliest stage of the menstrual cycle at which AM staining in the endometrium became apparent (Fig. 1C). Weak immunostaining for AM both in the epithelium of the endometrial gland and in the endometrial stroma was detected.

Mitotic activity is predominant in the early secretory phase in both the epithelial and stromal compartments, but such mitotic activity declines and terminates in the late secretory phase. Abundant immunostaining for AM was detected throughout the secretory phase with no appreciable difference in the early (Fig. 2A), mid (Fig. 2B), and late secretory phases (Fig. 2C). The epithelium of the endometrial glands and the endometrial stroma showed AM immunostaining throughout the secretory phase of the menstrual cycle.

On the other hand, the postmenopausal endometrium is characterized by markedly thin endometrium with a reduced number of glands lined by cuboidal or low columnar epithelium, and the stroma is basically fibrous with diminished cellularity. There was no detectable AM immunostaining in the epithelium of the endometrial glands, whereas the stromal compartment showed intense immunostaining for AM (Fig. 3).

Discussion

The endometrial tissue shows a complex pattern of growth and differentiation in every menstrual cycle, and this cyclical pattern is associated with varying levels of endocrine and local factors in the immediate environment *(26)*, which leads to histological changes in the endometrium. The endometrium can be viewed best on two parts; the endometrial glands and the surrounding stroma *(27)*.

In the present study, the immunohistochemical observations demonstrated the expression of AM in the epithelial and stromal compartments of the normal human endometrium with a notable change in cellular AM levels throughout the course of the menstrual cycle. Although several reports described the expression of AM in the endometrium *(24,25)*, no study has been conducted to elucidate the comparative expression of AM in the epithelial and stromal compartments in relation to the different phases of the menstrual cycle in the normal human endometrium. No immunostaining for AM in the epithelial cells of the endometrial glands and in the endometrial stroma was noted in both the early and mid proliferative phases. The earliest stage at which AM expression became apparent in the epithelium of the endometrial glands and in the endometrial stromal compartments was the late proliferative phase of the menstrual cycle. The immunostaining for AM was detected throughout the secretory phase of the menstrual cycle, both in the epithelium of the endometrial glands and in the stroma. Post menopausal endometrial tissues, on the other hand, showed abundant expression of AM only in the stromal compartment of the endometrium with negli-

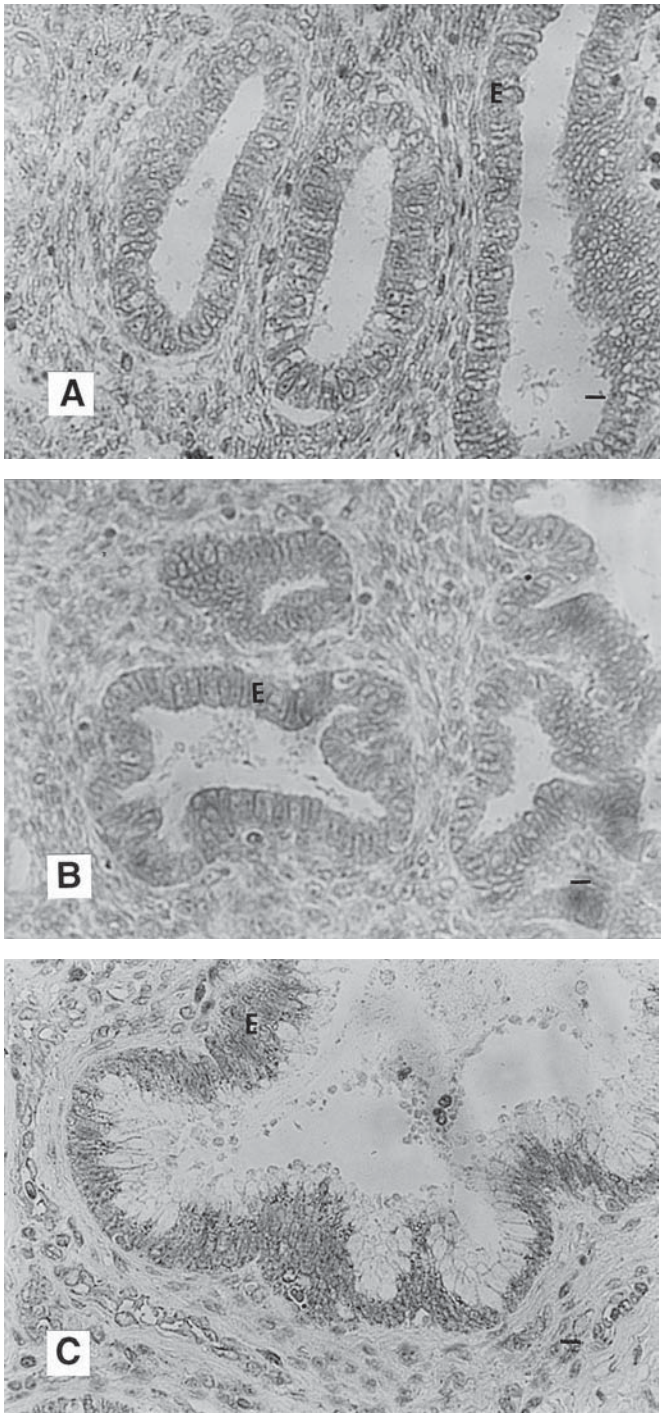

Fig. 2. Immunohistochemical localization of AM in the **(A)** early, **(B)** mid, and **(C)** late secretory phases of the endometrium. Abundant immunostaining was noted both in the epithelium of the endometrial glands and in the endometrial stroma throughout the secretory phase. E, epithelium of the endometrial gland; S, endometrial stroma. Bars represent 10 μm. Original magnification: ×400.

gible expression of AM in the epithelial compartment of endometrial glands.

Angiogenesis in the human uterus is an indispensable requirement to support the proliferation and repair of the

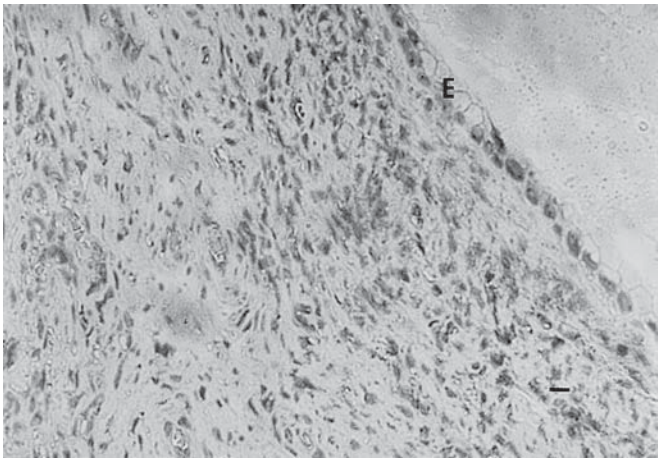

Fig. 3. Immunohistochemical localization of AM in the postmenopausal endometrium. Abundant immunostaining for AM was noted in the endometrial stroma, whereas there was negligible immunostaining for AM in the epithelium of the endometrial glands. E, epithelium of the endometrial gland; S, endometrial stroma. Bar represents 10 μm. Original magnification: ×400.

endometrium during the menstrual cycle. This provides a richly vascularized receptive endometrium for possible implantation and placentation. In addition, there are changes in vascular permeability throughout the menstrual cycle that facilitate progression of the thin, dense menstrual endometrium to the thickened highly edematous secretory endometrium. Although these changes are self-limited and presumably tightly regulated, the mechanisms underlying the growth and permeability of blood vessels in the endometrium are still unknown *(28,29)*. AM is a potent vasodilator *(12,13)* and is reported to be an angiogenetic factor *(24)*. In this context, AM expression became apparent in the late proliferative phase and became abundant in the secretory phase concomitantly with angiogenesis in the endometrium. This phase-related pattern of expression is consistent with the expression of vascular endothelial growth factor (VEGF) in the endometrium *(30,31)*. Shifren et al. *(30)* reported that the epithelial cells of endometrial glands express more VEGF than that of the stromal cells, and its mRNA level in the secretory phase is 3.6-fold more than that in the proliferative phase. The correlation in the expression of these two peptides, both of them known to act in the vasculature, is of significant interest.

However, vasodilatation is not the only biological function of AM. Several investigations done on AM revealed its cytoprotective function. AM was demonstrated to be an antimicrobial agent in the skin integument's protective barrier *(32)*. AM mRNA expression in cells lining the uterus, airways, and the gastrointestinal system may be related to the antimicrobial activity *(33)*.

The postmenopausal endometrial tissues, on the other hand, showed immunostaining for AM only in the stromal compartment of the endometrium with negative immunostaining in the epithelial compartment. It is reported that

fibroblasts, which are derived from a wide variety of tissues, express AM *(34)*. The expression of AM in the postmenopausal endometrial stroma could constitute an inherent expression by the fibroblasts that are abundant in the compact stroma. Cameron et al. *(33)* noted AM immunoreactivity in the columnar cells lining the uterus. The glands of the postmenopausal endometrium are lined by a single layer of flattened epithelial cells, and these glands are inactive owing to the diminished estrogen levels *(35)*. The expression of AM in the endometrium may be regulated by the changes in estrogen and progesterone levels. Further studies will be needed to investigate the role of AM in endometrial vascularization and in the stroma of the postmenopausal endometrium, as well as the possible participation of sex steroid hormones in regulating AM expression in the endometrium.

Materials and Methods

Tissue Specimens

Endometrial tissues were obtained from 18 women with regular menstrual cycles who underwent abdominal hysterectomy with uni- or bilateral salpingo-oophorectomy for a variety of gynecological conditions, including leiomyomata, ovarian cancer, adenomyosis, and cervical intraepithelial neoplasia. Postmenopausal endometrium was obtained from three postmenopausal patients who also underwent such operative intervention for cervical intraepithelial neoplasia. The collection of these tissues has been approved by the Institutional Review Board. These patients ranged in age from 26 to 73 yr, with a mean age of 44.2 yr. Informed consent was obtained from each patient before surgery for the use of endometrial tissues for immunohistochemical studies. Each specimen was examined by a pathologist for histological evaluation and dating of the endometrium. The day of menstrual cycle was determined by endometrial histological dating according to the method of Noyes et al. *(36)*. Endometrial tissues were graded into six categories: early proliferative (three cases), mid proliferative (three cases), late proliferative (three cases), early secretory (three cases), mid secretory (three cases), and late secretory phases (three cases) of the menstrual cycle. On histological examination, postmenopausal endometrium revealed atrophy of the endometrium.

The endometrial specimens obtained were fixed in 4% buffered neutral formalin, dehydrated and embedded in paraffin. Sections (4 μm in diameter) were deparafinized and examined using standard histological techniques.

Immunohistochemical Staining

Immunohistochemical staining was performed by the avidin-biotin immunoperoxidase method with the use of a polyvalent immunoperoxidase kit (Omnitag, Lipshaw, MI). A rabbit polyclonal antibody against human AM (Peninsula Laboratories, Belmont, CA) was used as the primary antibody. The anti-AM antibody was diluted 1:500 before use. The first incubation with the primary antibody was followed by the second incubation with avidin–horseradish peroxidase. Then, the chromogenic reaction was developed by incubating with a freshly prepared solution of tetrahydrochloride diaminobenzidine and hydrogen peroxide. The sections were counterstained with Harris hematoxylin, mounted with glycerine phosphate buffer solution, and examined microscopically.

The following control procedure was undertaken to ensure the specificity of the immunological reactions. Adjacent control sections were subjected to the same immunoperoxidase method, except that the primary antibody to AM was replaced by nonimmune rabbit serum at the same dilution as the specific antibody. In the aforementioned controls, no positive immunostaining was observed.

The intensity of the immunostaining was evaluated by two independent observers. It was graded as (–) for no immunostaining, (+) for weak but definitely detectable immunostaining, (++) for moderate immunostaining, and (+++) for intense immunostaining. The epithelial and stromal components of the endometrium were reviewed.

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