# The Contraction of Small Arteries in the Perimetrium by Presurgical Medication with the Luteinizing Hormone-releasing Hormone Agonist to Patients with Leiomyomas: An electron and immunoelectron microscopy

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# The Contraction of Small Arteries in the Perimetrium by Presurgical Medication with the Luteinizing Hormone-releasing Hormone Agonist to Patients with Leiomyomas : An electron and immunoelectron microscopy

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**Abstract** The remarkable contraction of small arteries occurred in the perimetrium of the uteri with leiomyomas by a presurgical medication with the luteinizing hormone-releasing hormone (LHRH) agonist (buserelin). The contracted vessels showed a significant increase in number of the Weibel-Palade (WP) bodies which occasionally underwent the degranulation and the discharge in a manner of exocytosis. By immunoelectron microscopy, localizations of endothelin (ET)-1 immunoreactive gold particles on the WP bodies as well as on cisterns of the rough endoplasmic reticulum (rER) and Golgi apparatus were pronounced in the buserelin-medicated group when compared to the non-medicated one. These findings indicate that the increase of the WP bodies storing ET-1, of which synthesis in the rER-Golgi system enhances, predominantly occurred in the small arteries in the perimetrium by the buserelin-medication prior to the hysterectomy. ET-1, released into the subendothelial layer, seems to play an important role in the contraction of the vascular media in a manner of paracrine.

## Introduction

Luteinizing hormone-releasing hormone (LHRH) agonists are now widely utilized for a medical treatment of uterine leiomyomas since this tumor is considered to be estrogen dependent and LHRH agonists decrease the secretion of estrogen from the ovary by down-regulation of gonadotropin receptors in the pituitary[6]. On the other hand, Morita et al.[9] reported that buserelin, a LHRH agonist, induces the contraction of the subserosal small artery and capillary of the uterus by light microscopy. However, to date, ultrastructural examinations of such a vasocontraction after a medical treatment with LHRH agonists have not been made.

Since the first description of Weibel-Palade (WP) bodies in the rat small artery[18], the role of this Golgi apparatus-derived inclusion has been analysed in two main aspects : A storage site of von Willebrand factor (vWf)[5][16][17] and GMP-140[1][7], and that of certain vasoactive substances such as histamine[2][3][4] and endothelin (ET)[11][12], a potent vasoconstrictor[19]. Co-existence of vWf and histamine in the same WP bodies was confirmed by the double immunolabeling technique[13]. A transient increase in number of the WP bodies in endothelial cells occurred in the perinatal rabbit[4] and human[5] umbilical veins during the vascular obliteration.

Sakamoto et al.[12] first demonstrated by immunoelectron microscopy that ET-1, synthesized in the rough endoplasmic reticulum (rER) and sorted in the Golgi apparatus, is stored in the WP bodies, and that the release of this peptide into both vascular lumen and subendothelium depends on the intracytoplasmic behavior of the WP bodies. On this basis, the involvement of WP bodies in the vasocontraction induced by LHRH agonists should be further elucidated.

On these grounds, the present study was designed to investigate ultrastructural and immunoelectron microscopic features, with an emphasis of those of the WP bodies, of vessels in the uterus with leiomyoma of patients who received buserelin for presurgical treatment.

## Materials and Methods

Tissue preparations: Samples for the present study were provided from six women who were performed total abdominal hysterectomy for symptomatic leiomyomas in our hospital. The patients consisted of three groups: The short-term medicated group (n=2, 46 and 49 years old) receiving the intranasal administration of buserelin acetate (Hoechst Japan Ltd.) at doses of 900 $\mu$g/day for 4 and 6 weeks prior to operation, the long-term medicated group (n=2, 35 and 41 years old) receiving the same doses of the drug for 24 weeks, and the non-medicated group (n=2, 39 and 45 years old).

Specimens isolated from the perimetrium at least 5 cm distant from each tumor margin were fixed in 2% paraformaldehyde-1% glutaraldehyde in 0.1M phosphate buffer for 2 hr at 4°C and postfixed in 1% osmium tetroxide in the same buffer for 2 hr at 4°C. After dehydration in graded concentrations of ethanol, they were embedded in epoxy resin. For electron microscopy, ultrathin sections mounted on nickel grids were stained with 5% uranyl acetate and lead citrate, and examined in a JEM 1210 electron microscope.

Quantitative evaluation of Weibel-Palade bodies: The number and the area of the WP bodies per 100 $\mu m^2$ endothelial cells area were carried out with an image analysing device (Nikon Cosmosone IS). Photographs of randomly selected 20 cells from each group were taken at X10,000 and printed at a final magnification of X60,000. The number of the WP bodies were directly counted in electron micrographs, and the area of each endothelial cell and total area occupied by the WP bodies per cell were measured. These data are shown in Table 1 expressed as mean ± SEM. Statistical comparisons between the medicated and non-medicated groups and between the short-term and long-term medicated groups were made using Student's t-test for unpaired comparisons.

Immunoelectron microscopy: All procedures were done at room temperature. Ultrathin sections were etched with saturated sodium metaperiodate for 40 min and placed on drops of 1.0% egg albumin (EA) in a phosphate-buffered saline (PBS) for 15 min to absorb non-specific proteins. The grids were incubated for 4 hour in mouse anti ET-1 serum (Yamasa-shoyu Co., Chiba, Japan) diluted at 1 : 1,000 in 0.1% EA-PBS. After rinsing in PBS, the grids were reacted to goat anti-mouse IgG-coated 15 nm colloidal gold (Ultra Biosols, Liverpool, UK) diluted at 1 : 100 in 0.1% EA-PBS for 1hr. The grids were slightly washed in PBS and then in distilled water, stained with 5% uranyl acetate and lead citrate, and examined in the electron microscope. The specificity of the immunoreactions was confirmed by substituting the primary antiserum either with normal mouse serum or PBS.

## Results

Electron microscopy

Figure 1 shows a profile of the small artery in the perimetrium of the non-medicated group. Endothelial cells of the vessel occasionally

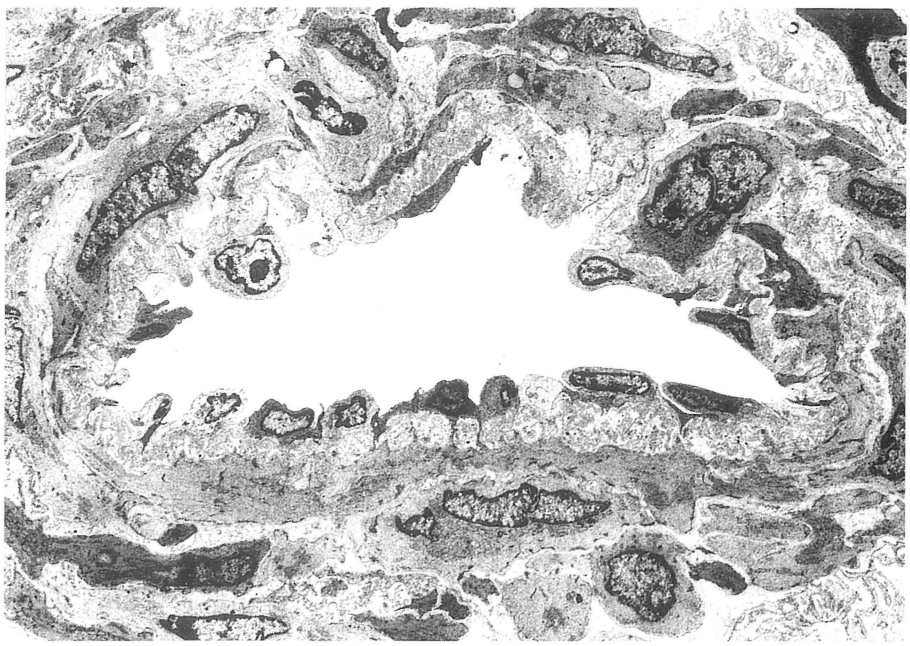

**Fig. 1** A profile of small artery in the perimetrium of the non-mendicated group. ×2,000.

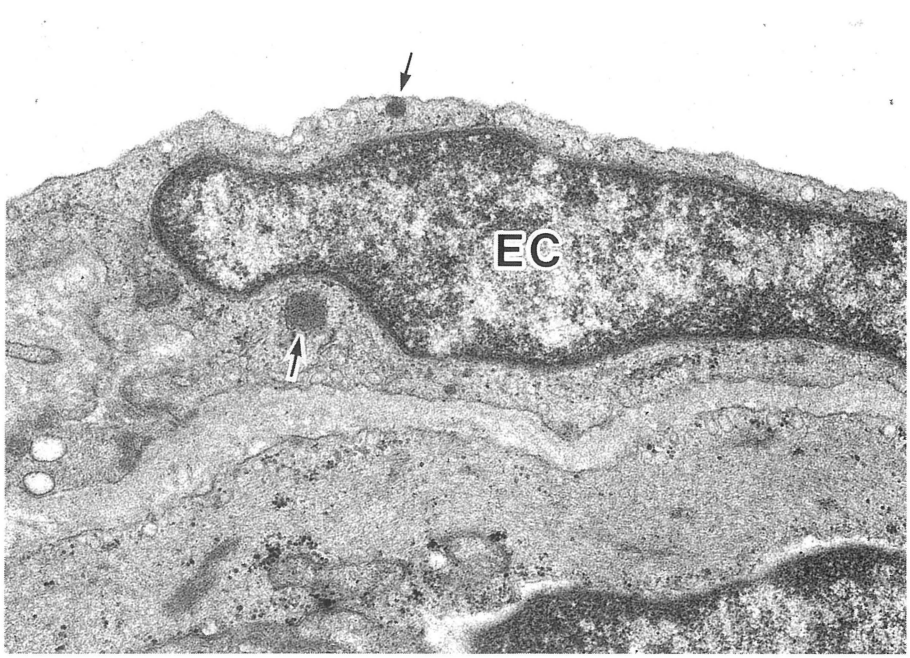


**Fig. 2** An endothlial cell (EC) contains a few WP bodies (arrows) in the small artery of the non-medicated group. ×20,000.

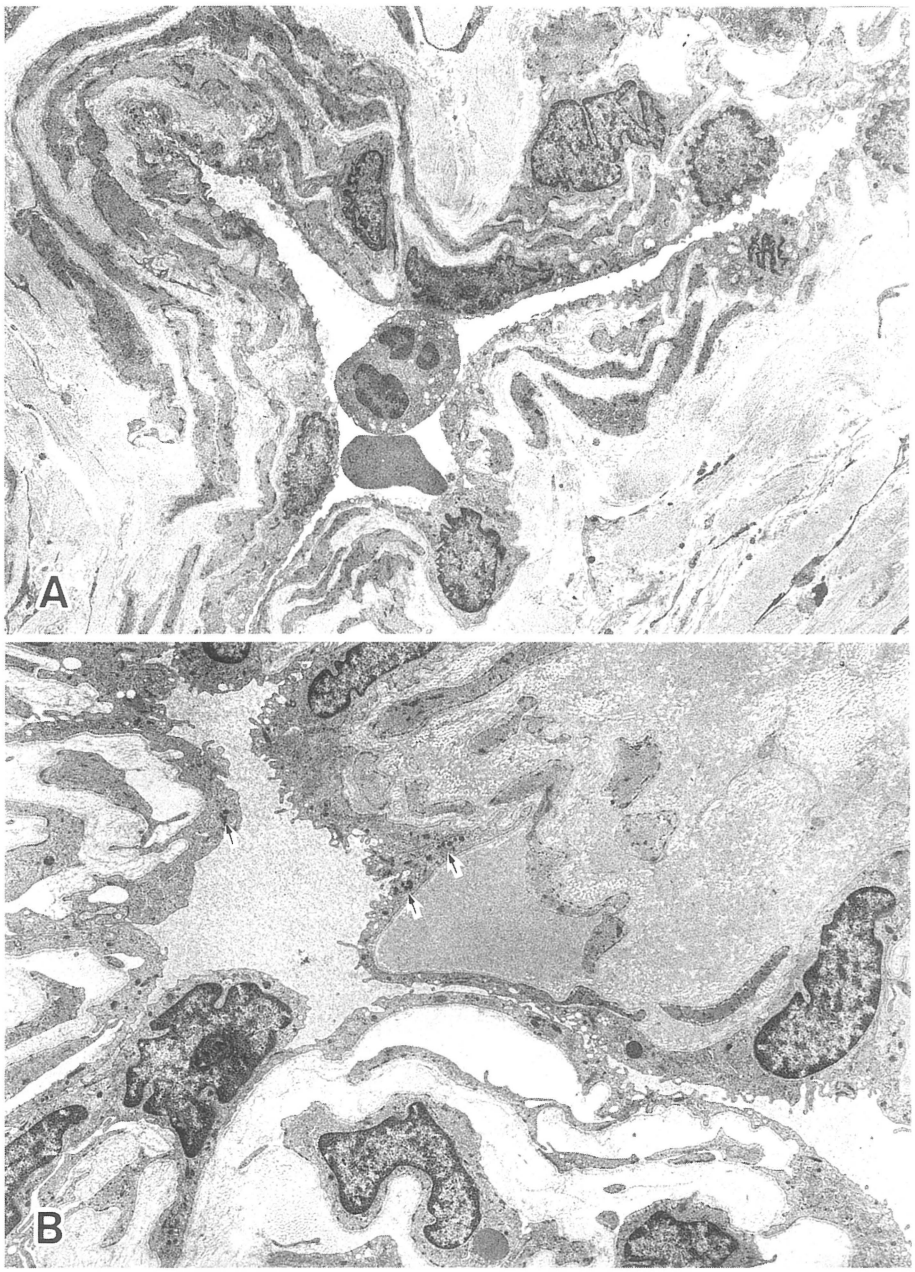


**Fig. 3** The contraction of small arteries of the short-term (A) and the long-term (B) medicated groups. WP bodies increase in number (arrows). A : ×2,000. B : ×3,800.

contained a few WP bodies (Fig. 2). After administration of buserelin, the marked vasocontraction predominantly occurred in the vessels of both short-term (Fig. 3A) and long-term (Fig. 3B) medicated groups. A significant increase in number of the WP bodies occurred in both groups (Figs. 4A and 4B). Some WP bodies were swollen (Fig. 4B) and were in a close apposition to the basal plasma membrane of the endothelial cell in a manner suggestive of

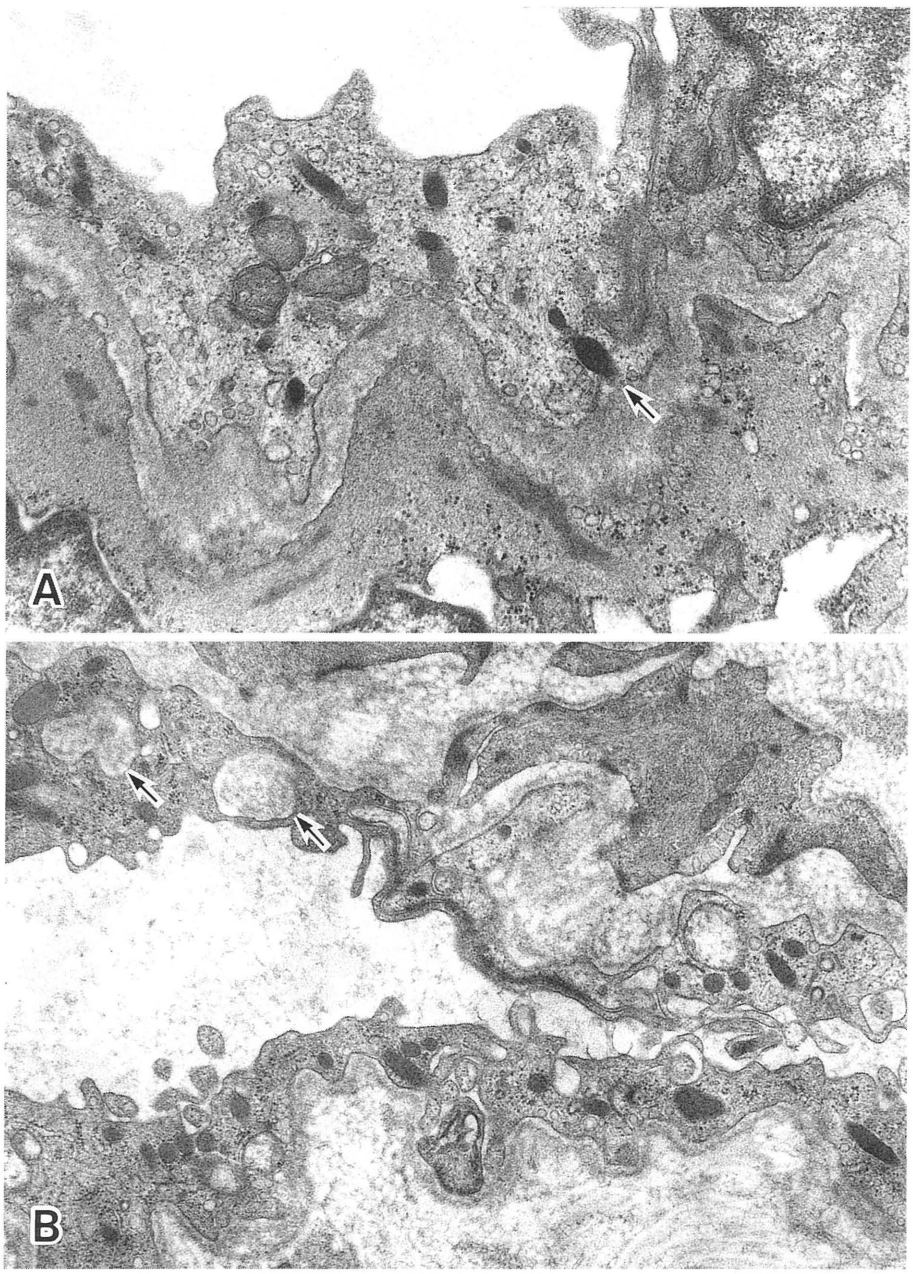


**Fig. 4** The increase of WP bodies in endothelial cells of small arteries of the short-term (A) and the long-term (B) medicated groups. A close apposition of a WP body to the basal plasma membrane (arrow in A) and the swelling (arrows in B) of the WP bodies are seen. A and B: ×20,000.

exocytosis (Fig. 4A).

### Quantitative determination of WP bodies

The quantitative data are summarized in Table 1. As shown in this table, the number of WP bodies and the total area of WP bodies per 100 $\mu m^2$ endothelial cell area of the medicated groups significantly increased ($p<0.01$), respec-

**Table 1** Quantification of Weibel-Palade (WP) bodies by means of image analysis

| | Number of WP bodies per 100 $\mu m^2$ EC area | Total area of WP bodies ($\mu m^2$) per 100 $\mu m^2$ EC area |
|---|---|---|
| Non-medicated group (20 cells from 2 patients) | 111.6±20.2 | 0.63±0.22 |
| Short-term medicated group (20 cells from 2 patients) | 442.8±61.2* | 2.02±0.32* |
| Long-term medicated group (20 cells from 2 patients) | 705.0±160.2* | 3.09±0.50* |

All data are expressed as means ± SEM. *$p<0.01$ EC: endothelial cell

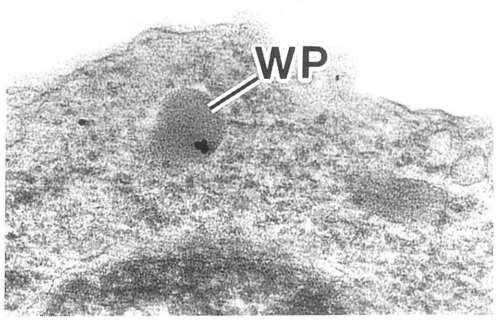


**Fig. 5** Immunoreactions of ET-1 on a WP body (WP) in an endothlial cell of small artery of the non-medicated group. ×55,000.

tively, when compared to that of the non-medicated group. There are no significant differences between the short-term and long-term medicated groups.

### Immunoelectron microscopy

In the non-medicated group, a few ET-1 immunoreactive gold particles were occasionally localized on the WP bodies (Fig. 5), but the gold particles were rarely found on cisterns of the rER and Golgi apparatus. In contrast, a considerable number of the gold particles were localized on such cisterns as well as on the WP bodies in both short-term and long-term medicated groups (Figs. 6 and 7). Few or no immunoreactions were observed in both non-medicated and medicated groups when the primary antiserum was replaced by either normal mouse serum or PBS as negative controls.

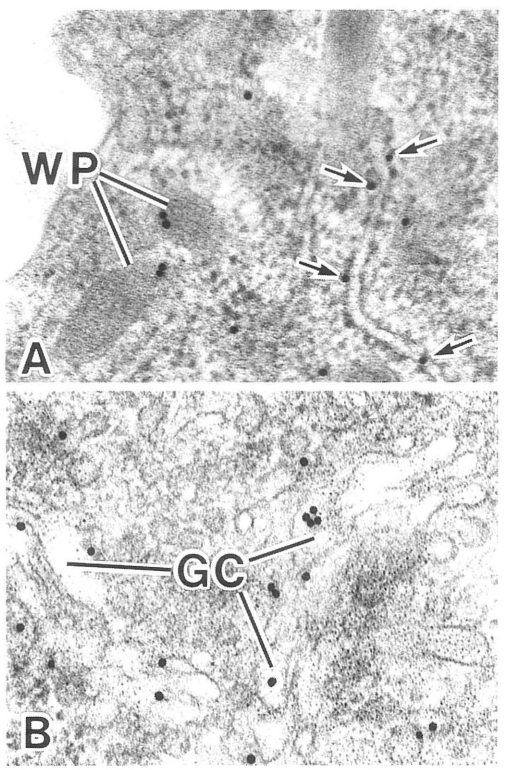


**Fig. 6** Endothelial cells of small arteries of the short-term medicated group. A: Immunoreactions of ET-1 on a cistern of the rER (arrows) and on WP bodies (WP). B: Immunoreactions of ET-1 on cisterns of the Golgi apparatus (GC). A and B: ×55,000.

### Discussion

It is now widely accepted that LHRH agonists suppress estrogen secretions from the ovary by down-regulation of gonadotropin rece-

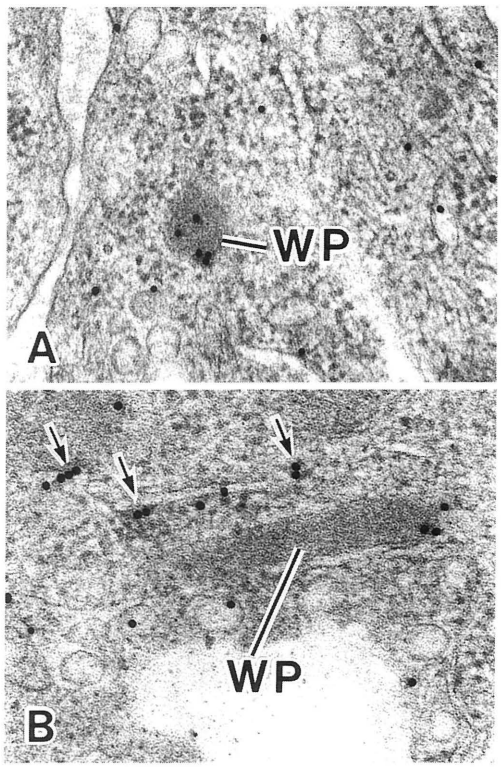


**Fig. 7** Endothelial cells of the small arteries of the long-term medicated group. A: Immunoreactions of ET-1 on a WP body (WP). B: Immunoreactions of ET-1 on a WP body (WP) and on cisterns of the rER (arrows). A and B: ×55,000.

ptors in the pituitary, resulting in the decrease of the plasma estrogen levels[6]. Since estrogen acts on blood vessels as a vasodilatative factor[8], vasocontractions induced by LHRH agonists have been considered to be mainly due to the decrease of the plasma estrogen levels.

However, the decrease of the plasma estrogen level seems to be unlikely in the short-term medicated group in our samples judging from the previous data[14)15)]. Morita et al.[9] reported the contraction of the uterine vessels is induced by the buserelin medication and suggested that buserelin may act directly on the uterine vessels as a vasoconstrictive factor, independent of the decrease of the plasma estrogen level.

The contraction which predominantly occurs in small arteries in the perimetrium by the present observation revealed two significant ultrastructural and immunocytochemical alterations: The increase in number of the WP bodies and the enhancement of ET-1 synthesis in the rER-Golgi system. As shown in Table. 1, the number of the WP bodies and the total area occupied by the WP bodies per 100 $\mu m^2$ endothelial cell area in the medicated groups significantly increased when compared to those of the non-medicated group by quantitative evaluations. These phenomena argue for the data in the human and rabbit umbilical veins during the vascular obliteration[4)5)].

ET-1 immunoreactive gold particles were occasionally found on the WP bodies of the small arteries in the non-medicated group. This implies that the WP bodies of these vessels are involved in the storage of ET-1 under normal conditions as in the case of other vessels[10)11)12)]. However, the immunoreactions on the rER-Golgi system and on the WP bodies were much more pronounced in the medicated groups. These findings suggest that buserelin induces the enhancement of both synthesis and sorting of ET-1 in the rER-Golgi system and the storage of ET-1 in the WP bodies. The degranulation and the extracellular discharge in a manner suggestive of exocytosis of the WP bodies from the endothelial cells into the subendothelium were indicated from our electron micrographs of the medicated groups. Since the discharge of the WP bodies through the basal plasma membrane of endothelial cells occurs under normal condition[12] and the existence of ET-1 receptors on the sarcolemma of vascular smooth muscle cells is well known, it is reasonable to assume that the WP bodies storing ET-1 play the crucial roles in the contraction of the vascular media in a manner of paracrine.

The present study raises questions of whether other vasoactive substances included in the WP bodies are also involved in our buserelin-induced vasocontractions, whether

the enhancement of ET-1 synthesis primarily induces an increase in number of the WP bodies since the increase also occurred during the vascular obliteration[4)5)], and whether buserelin selectively acts on the small artery in the perimetrium in a receptor-mediated mode since the vasocontraction predominantly occurred in the small artery. To solve these problems, further studies using experimental animals are now in progress in our laboratory.

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(和文抄録)

# 黄体化ホルモン放出ホルモン主動薬（LHRH agonist）による子宮外膜小動脈血管収縮の電顕ならびに免疫電顕的解析

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子宮筋腫により腹式単純全摘出術を施行した子宮について，LHRH agonistであるブセレリンの術前投与が非筋腫部の子宮外膜血管に及ぼす影響を電子顕微鏡とエンドセリン-1（ET-1）を一次抗体に用いた免疫電顕で検索した。症例の内訳は，2例が非投与群，2例が900 μg/日量のブセレリンの経鼻腔投与を4週間と6週間行った短期投与群，2例が同量のブセレリンを24週間投与した長期投与群であった。

投与群においては，子宮外膜の小動脈に著明な血管収縮が認められ，その血管内皮細胞のWeibel-Palade（WP）小体が非投与群に比べて有意に増加し（$p<0.01$），かつその内皮下層への放出像が著明であった。投与群においては，ET-1免疫陽性金粒子の増加したWP小体上への局在と粗面小胞体とゴルジ装置への局在が顕著であったのに対し，非投与群では，それらの局在性はまれであった。

以上の所見は，ブセレリン投与がET-1貯蔵に関与するWP小体の増加と細胞外放出を促進させ，LHRH agonist誘発エストロジェン血中濃度の低下による血管収縮機序とは直接的には関係のない機序によって子宮外膜小動脈の収縮が誘発されたことを示唆する。