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Effects of relaxin on the microvasculature of mouse mammary gland

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Summary. The effects of RLX on the microvasculature of the mouse mammary gland are reported. RLX (pure porcine standard NIH-RXN-P1) at a dose of 3 GPU was administered subcutaneously to virgin adult mice ovariectomized 12 days before.

The mammary glands were removed 18-20 h after RLX injection and their examination by light microscopy did not reveal any substantial growth-response to the hormone.

Histology and morphometry indicated striking dilation of microvessels, especially capillaries, and electron microscopy revealed an increase in the micropinocytotic vesicles, thus suggesting enhanced transendothelial transport of substances.

Such phenomena, which were independent of a release of granules by mast cells, may represent an important component of the mammotrophic action of RLX.

Key words: Relaxin, Mammary gland, Microvasculature

Introduction

In recent years several different roles have been claimed for relaxin as a hormone acting both systemically and locally (Bryant-Grenwood, 1982). The availability of purified preparations of RLX extracted from the ovary of pregnant pigs and rats (Sherwood and O'Byrne, 1974; Sherwood, 1979; Walsh and Niall, 1980) has enabled the action of this hormone to be assayed experimentally, thus providing insight into its role in controlling the physiology of reproductive organs in both the male and female (Yki-Järvinen et al., 1983 a, b) and also revealing other unexpected effects such as those on microvasculature, as indicated by our recent studies on the rat mesocaecum (Bigazzi et al., 1986). In fact, RLX locally administered was found to cause consistent vasodilation of mesocaecal microvessels, especially venules, and to counteract vasoconstriction due to norepinephrine and promethazine.

A conspicuous enlargment of microvessels, including blood capillaries, has also been observed by us (Bani and Bigazzi, 1984) in the mouse mammary gland undergoing growth induced by systemically administered RLX after priming with estrogen and in the pigeon crop sac mucosa following local administration of the hormone (Bani et al., 1987). The increase in the microvascular compartment could be considered by us as a collateral phenomenon related to the whole mammary growth promoted by the hormonal treatments as well as by other authors in the growing uterus of estrogen-primed rats who received RLX (Vasilenko et al., 1986). However, the changes observed in the microvessels of mesocaecum of male rats, to which RLX was given locally and without any pre-treatment with other hormones, raise the possibility that RLX has a specific dilatory effect on the microvasculature and independent of a growth response of the assayed organ.

The aim of this study was to investigate whether RLX influences per se on the diameter of microvessels and the cytological components involved in transendothelial transport of the exchange vessels in the mouse mammary gland following systemic administration and without a pre-treatment with estrogen, a condition in which our previous findings (Bani et al., 1985; 1986) failed to demonstrate any substantial growth of the mammary parenchyma.

Materials and methods

Ten female albino virgin Swiss mice, eight weeks old and weighing 28-32g were used in this study. The animals were ovariectomized bilaterally at laparotomy. Five were sacrificed 12 days after surgery without any

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hormonal treatment and used as controls. Five received 1 μg (3 GPU) porcine RLX standard (NIH-RXN-P1) 12 days after surgery and were sacrificed 18-20 h after RLX administration.

On sacrifice, the two most caudal pairs of the ten mammary glands, i.e. the abdominal and inguinal pairs, were removed from each animal. One gland of each pair was fixed in Bouin's fluid, embedded in paraffin and cut into 5 μm thick sections, which were stained with hematoxylin and eosin and toluidine blue. The other gland of each pair was fixed in cold 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at room temperature, and postfixed in 1% $0s0_4$ in 0.1 M phosphate buffer, pH 7.4, at 5° C. Dehydration was performed in graded acetone series and embedded in Epon 812. From the specimens processed for electron microscopy semithin sections 1-2 μm thick were obtained and stained with toluidine blue - Na tetraborate. Ultrathin sections were also cut, which were stained with uranyl-acetate and alkaline bismuth subnitrate (Riva, 1974), and examined under a Siemens Elmiskop 102 electron microscope at 80 kV.

Morphometry was performed on semithin sections from glutaraldehyde, and $0s0_4$ – fixed, Epon – embedded specimens in which it was easy to recognize the different anatomical portions of the microvessels from the histological structure of their walls (see Simionescu and Simionescu, 1984).

Morphometrical evaluations were performed on the periductal small vessels by a computer-assisted method, using a digitizing tablet with resolution of 0.025 mm (Numonics 2210, Numonics corp., Lansdale, PA, USA) interfaced with an Apple IIGS computer (Apple Computer Inc., Cupertino, CA, USA) with 1256 Kbyte memory RAM, a disk driver 3.5" (800 Kbyte formatted), a color monitor and an Apple Imagewriter II color graphic printer.

The digitizer sends to the computer (via an Apple super serial card) a signal representing the X, Y coordinate position of the pen stylus as it is moved around the profile being measured.

The software, written in 65C816 assembler and C languages by one of the authors (S. Bianchi), converts the coordinate data into geometric measurements of area, perimeter, diameters (maximum, minimum and average diameters) and the form factor of the digitizer profile. These geometric parameters were calculated according to the algorithms proposed by Mize (1985).

Results

Light microscopy revealed that microvessels were more frequently located in the connective tissue enveloping the major mammary ducts, running along the major axis and at the base of the nipple, and near the small ducts originating from the lactiferous sinus and expanding under the areola. In both these sites the lumina of the microvessels appear narrow in the control mice (Fig. 1) and strikingly enlarged following RLX administration (Fig. 2).

The morphometrical analysis of the dimensions of microvessel lumina revealed that a significant vasodilation occurs in arterioles, capillaries and postcapillary after the hormonal treatment venules after the hormonal treatment. In fact, the mean diameters in the RLX-treated mice compared with controls were for arterioles (Fig. 3): 34.48 +/– 2.2 μm versus 22.89 +/– 1.4 μm (% increase 52.39), for capillaries (Fig. 4): 15.94 +/– 0.37 μm versus 8.90 +/– 0.2 μm (% increase 78.95), for postcapillary venules (Fig. 5): 35.24 +/– 1.1 μm versus 21.15 +/– 0.8 μm (% increase 66.56).

Electron microscopy of the endothelium of the small exchange vessels (capillaries and postcapillary venules) revealed some changes in cytological structures related to transendothelial transport in the RLX-treated mice compared with the control counterparts.

In the control mice the endothelium of the exchange vessels showed ultrastructural features quite similar to those usually reported for the continuous capillaries and venules. It consisted of a single layer of squamous epithelial cells (Fig. 6) joined by intercellular junctions characterized by closely apposed membranes running parallel with an interposed clear gap up to 60 nm wide and points of membrane fusion (occluding junctions) toward the luminal front of the endothelium (Fig. 6, insert). Small bundles of cytofilaments could be seen converging towards the points of membrane fusion. The cytoplasm of the endothelial cells contained the usual complement of organelles, consisting of small quantities of rough endoplasmic reticulum, free ribosomes, small Golgi zones, slender mitochondria and moderate numbers of pinocytotic vesicles of average 60-80 nm which appeared either open on the two endothelial cell fronts or free in the cytoplasm (Fig. 7).

In the RLX-treated mice the endothelial lining of the exchange vessels often became very thin. No substantial changes occurred in the organelles of the endothelial cells (Fig. 8) or in the intercellular junctions (Fig. 9), whereas a conspicuous increase was found in the number of pinocytotic vesicles. These vesicles often formed clusters extending from the luminal to the abluminal faces (Fig. 8). Also, large vacuoles apparently formed by coalescent pinocytotic vesicles were frequently seen (Fig. 9) and rows of pinocytotic vesicles (Fig. 10) appeared to open simultaneously on both the endothelial fronts probably allowing direct communication between the lumen of the vessel and the interstitial compartment.

Examination of mast cells in sections stained with toluidine blue revealed similar pictures in the controls (Fig. 11) and the RLX-treated mice (Fig. 12), no consistent difference being found in the number of these cells or in their content of metachromatic granules, which were commonly tightly packed inside the cytoplasm and did not undergo substantial exocytosis processes.

Discussion

These morphometrical and histological findings indicate that RLX induces morphological changes in the microvasculature of the mouse mammary gland. In fact,

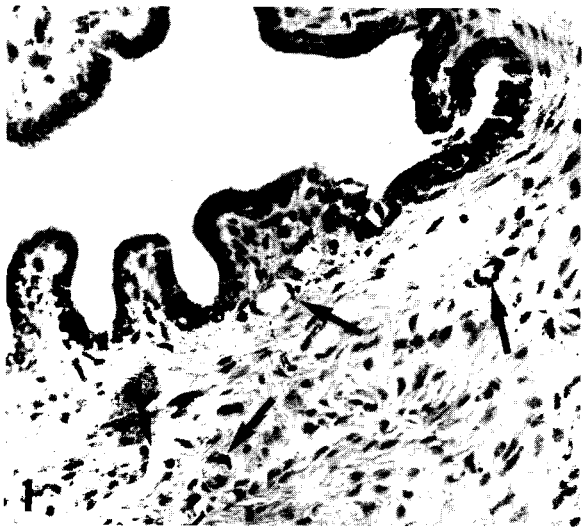


Fig. 1. Mouse mammary gland. Control. Some blood capillaries can be seen with narrow lumina (arrows) in the connective tissue near a large mammary duct. Hematoxylin and eosin. × 320

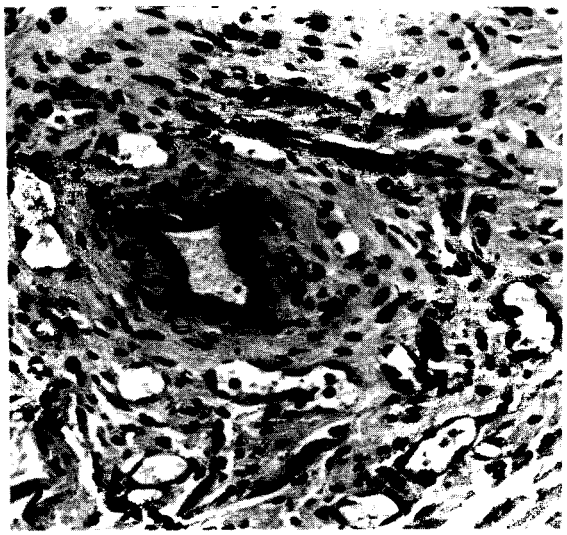
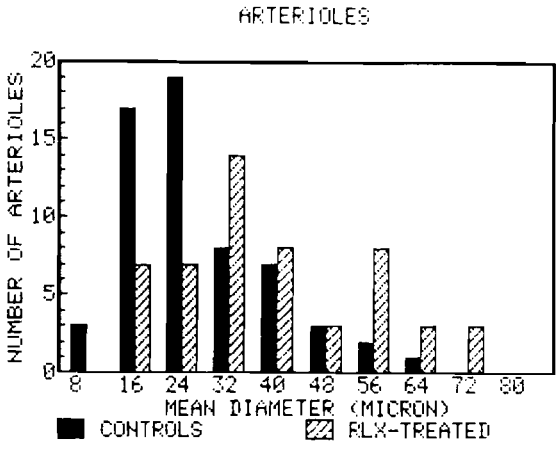


Fig. 2. Mammary gland from a mouse treated with 3 GPU RLX. Strikingly dilated blood capillaries can be seen in the connective tissue surrounding a large mammary duct. Hematoxylin and eosin. × 320



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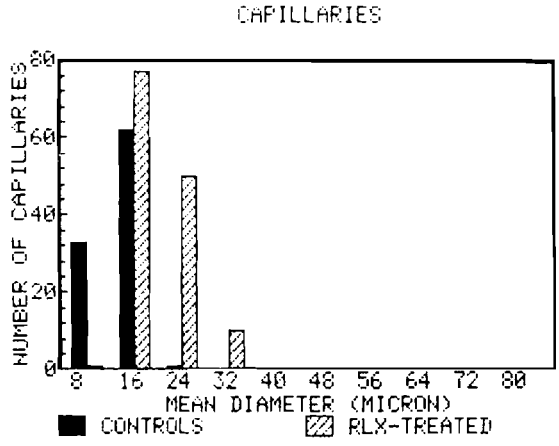

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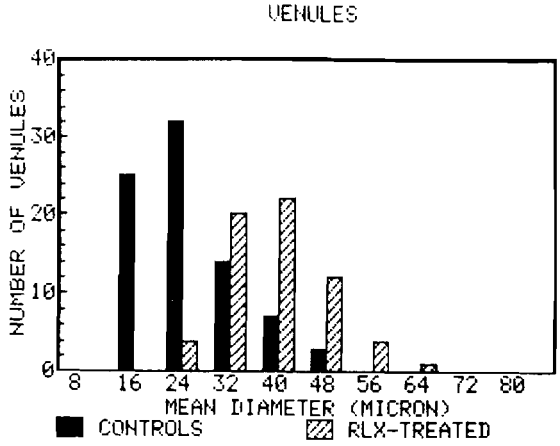

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Fig. 3. Histogram of the mean diameters of arterioles in controls and RLX-treated mice. F test significant for $P < 0.1$

Fig. 4. Histogram of the mean diameters of capillaries in controls and RLX-treated mice. F test significant for $P < 0.1$

Fig. 5. Histogram of the mean diameters of venules in controls and RLX-treated mice. F test significant for $P < 0.1$

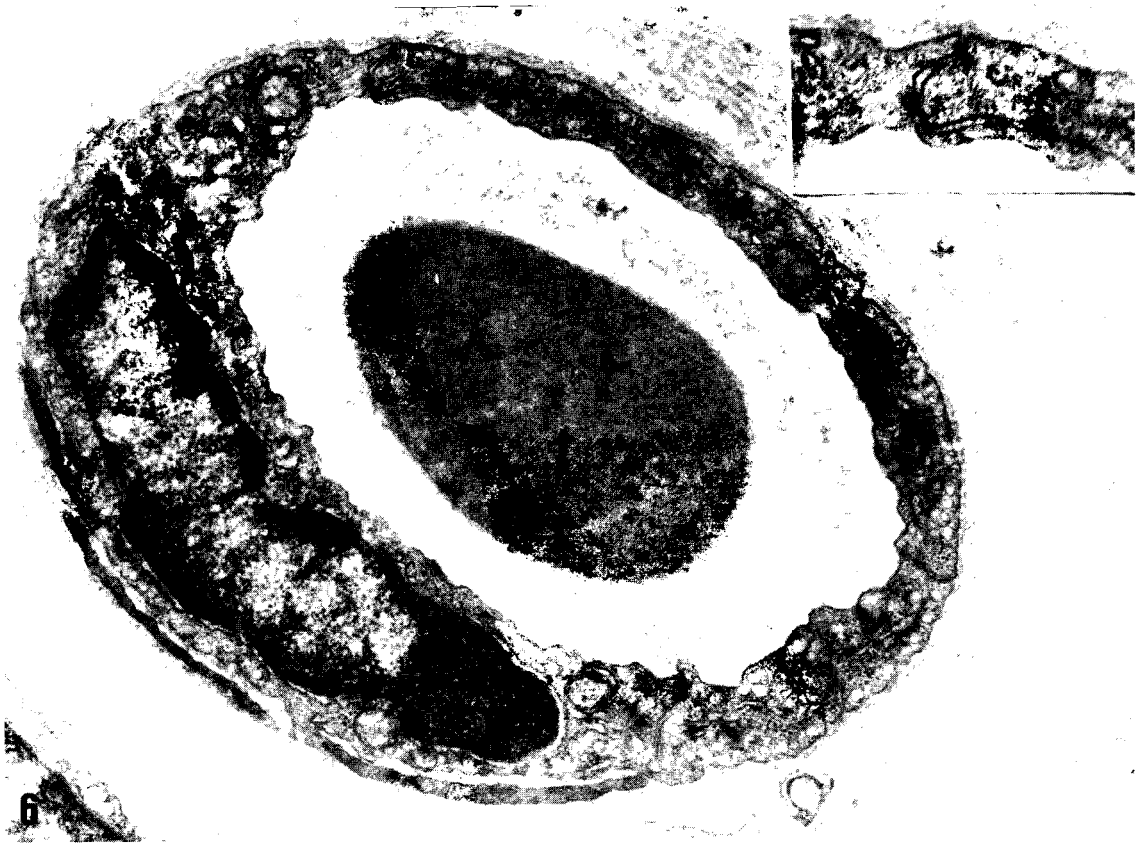

Fig. 6. Mouse mammary gland. Control. A blood capillary near a mammary duct. Endothelial cells form a continuous lining and contain the usual set of organelles. × 25,200. In the insert a detail of an intercellular junction can be seen, with membranes running parallel and occlusion of the intercellular space towards the luminal face. × 50,000

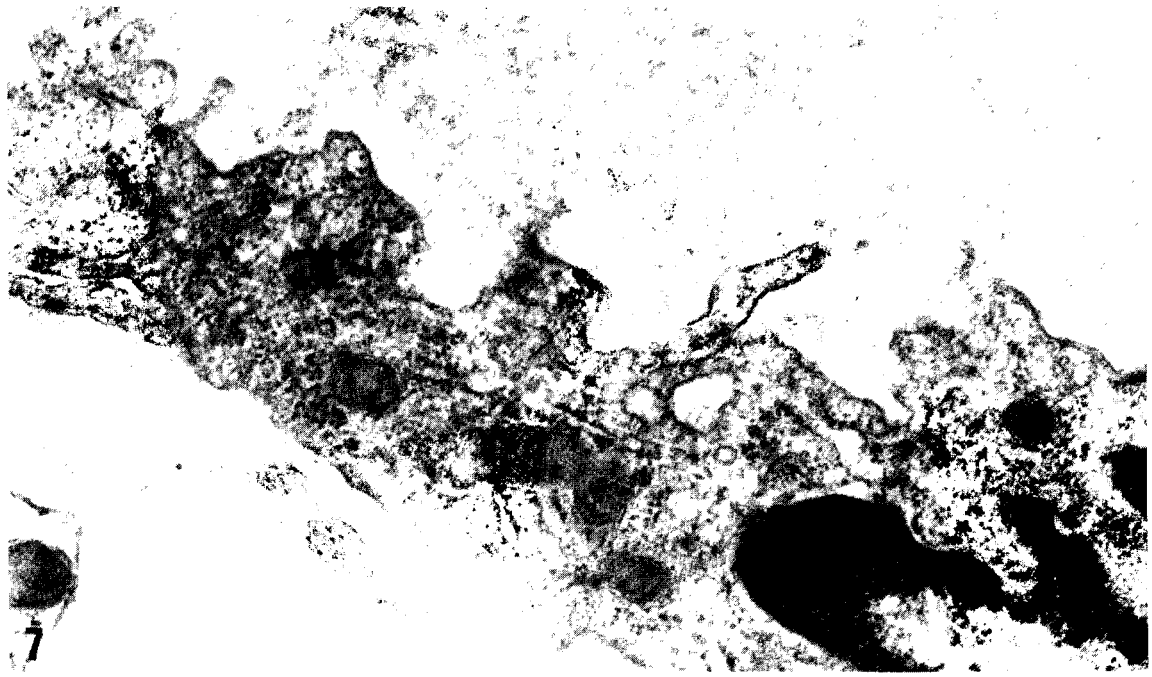

Fig. 7. Mouse mammary gland. Control. Portion of endothelial cell of a blood capillary near a mammary duct. Pinocytotic vesicles are sparse. × 37,500

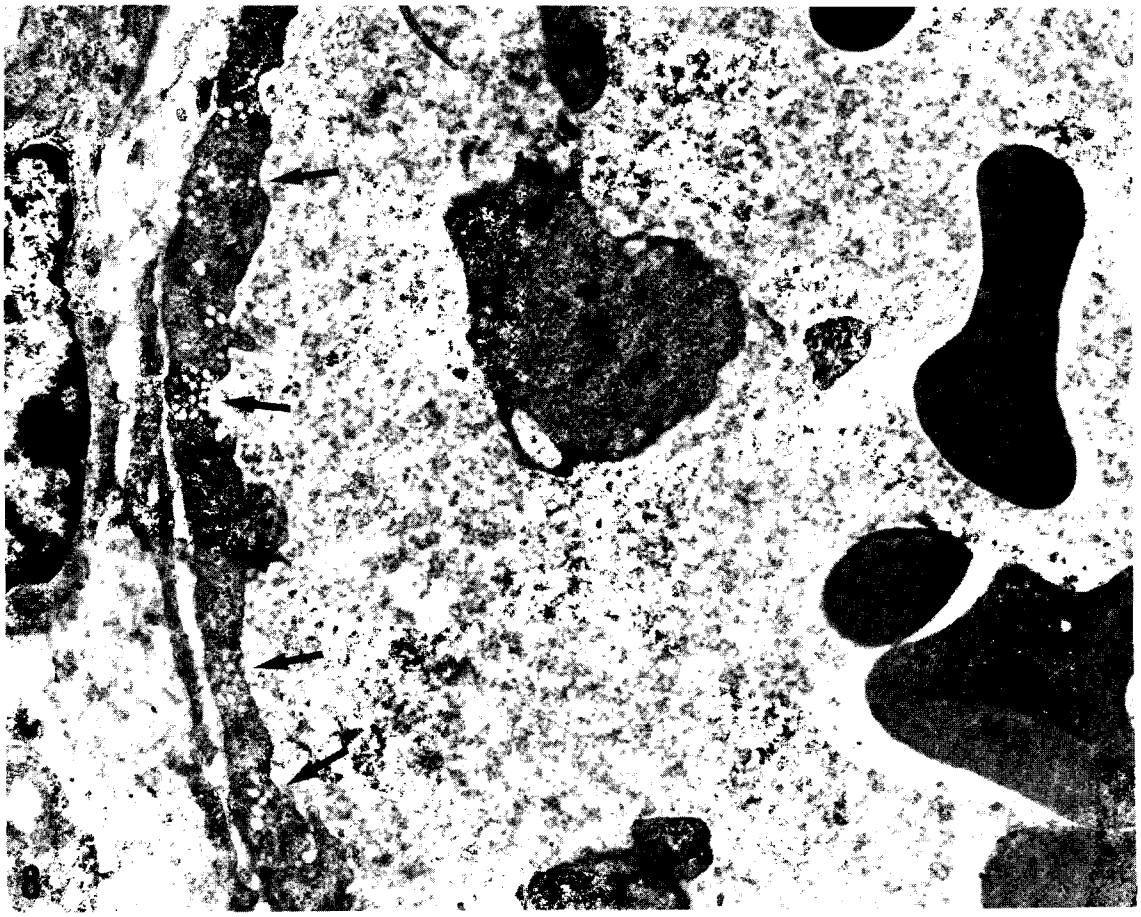

Fig. 8. Mammary gland from a mouse treated with 3 GPU RLX. A dilated blood capillary can be seen with flattened endothelial cells provided with numerous clusters of pinocytotic vesicles (arrows). × 9,000

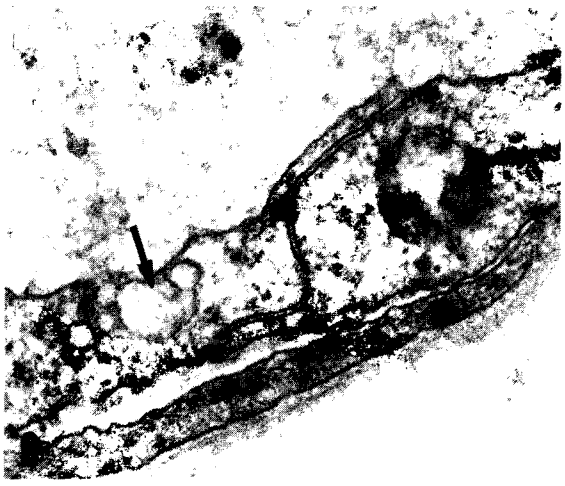

Fig. 9. Mammary gland from a mouse treated with 3 GPU RLX. A portion of blood capillary endothelium can be seen with normal intercellular junction and a cluster of pinocytotic vesicles coalescing into a large vacuole (arrow). × 37,500

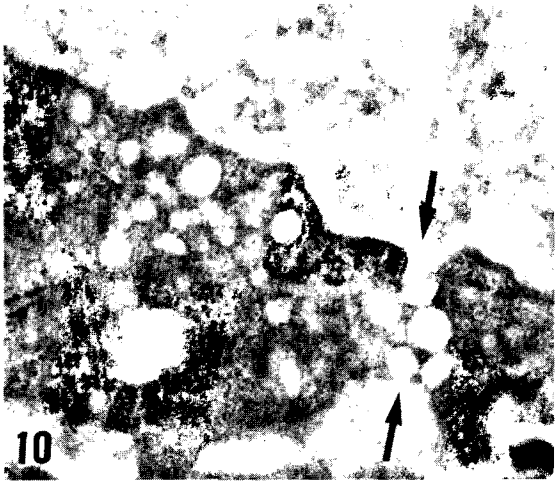

Fig. 10 Mammary gland from a mouse treated with 3 GPU RLX. A detail of a blood capillary endothelium showing a row of pinocytotic vesicles extending from the luminal to the abluminal face (arrows). × 37,500

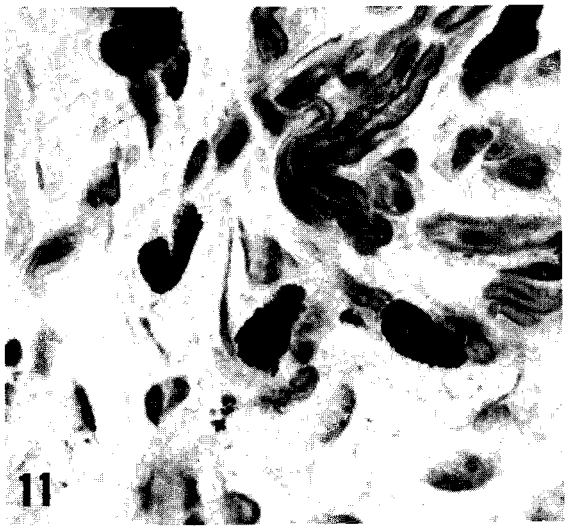

Fig. 11. Mouse mammary gland. Control. Mast cells with cytoplasm filled with metachromatic granules. Toluidine blue. × 800

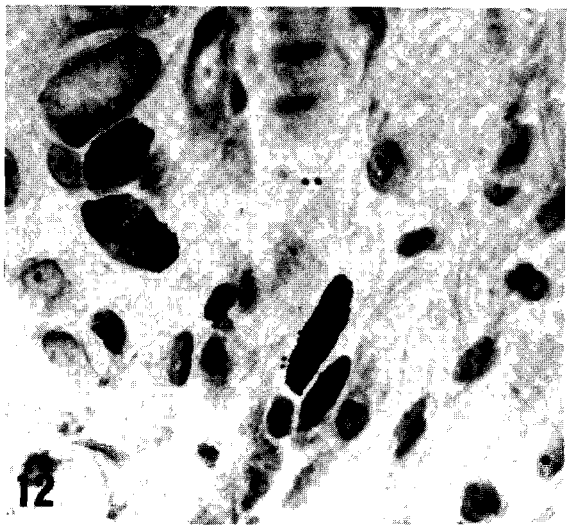

Fig. 12. Mammary gland from a mouse treated with 3 GPU RLX. Mast cells do not show any sign of granule release. Toluidine blue. × 800

RLX causes striking dilation of the entire microvascular bed, especially capillaries. Such an effect is probably due to an inhibitory action of the hormone on the contractile apparatus of the cells forming the walls of the microvessels. Indeed, the smooth muscle cells of the arterioles, that in the controls showed nuclei with scalloped contours, following RLX had nuclei with linear profiles, as occurs in the non-contracted smooth muscle cells. Moreover, our recent findings on the blood capillaries of the pigeon crop sac mucosa indicated that, along with dilation, a rearrangment of the endothelial microfilaments occurs. Such effects of RLX are in agreement with previous reports on the well-known action of this hormone in inducing relaxation of smooth muscle cells of uterus and cervix in vivo (Bryant-Greenwood, 1962) and of myometrial cells in vitro (Hsu and Sanborn, 1986; Ramachandra Rao and Sanborn, 1986).

The effects of RLX in the experimental condition here studied, are independent of a growth response of the organ to the hormone, thus confirming a specific effect of RLX on the microvasculature, in agreement with the results of our previous studies on mesocaecum in vivo (Bigazzi et al., 1986). Moreover, electron microscopy reveals in the endothelium of the exchange vessels an increase in the pinocytotic vesicles which are commonly considered to be involved in the transendothelial transport of macromolecules (see Simionescu and Simionescu, 1984).

The lack of enhanced release of mast cell granules in the RLX-treated animals as compared with controls strongly suggests that microvessel changes are not mediated by the vasoactive substances of mast cells.

The positive influence of RLX on both microvessel dilation and transendothelial transport, that obviously results in increased blood supply and enhanced blood-tissue exchanges, is to be considered as an important component of the trophic action of this hormone, which has previosly been documented by us in the mammary glands of ovariectomized, estrogen-primed mice (Bani et al., 1985; 1986; Blanchi et al., 1986; Bani Sacchi et al., 1987) and in the pigeon crop sac (Bani et al., 1987), and by others in the uterus (Vasilenko et al., 1986).

Moreover, the present findings may account for a previously unexplained clinical observation, that RLX causes remission of symptons in patients with obliterative peripheral arteriopathy (Casten et al., 1960).

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