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# The effect of somatostatin analog octreotide on diethylstilbestrol-induced prolactin secretion, cell proliferation and vascular changes in the rat anterior pituitary gland

**M. Pawlikowski, J. Kunert-Radek, M. Grochal, K. Zieliński, and A. Kulig**

Institute of Endocrinology, Medical University of Lodz and Departments of Neurosurgery and of Pathomorphology, Military School of Medicine, Lodz, Poland

**Summary.** The effects of diethylstilbestrol (DES) and of long-acting somatostatin analog, octreotide (SMS) on the rat anterior pituitary microvasculature have been studied by means of computer-assisted image analysis. Additionally, the effects of DES and SMS on prolactin secretion and anterior pituitary cell proliferation have been studied, as well. The vascularization was visualized using Selye's method modified by Poely et al. (1964). The prolactin serum levels were estimated by radio-immunoassay. The proliferation indices were assessed using bromodeoxyuridine incorporation assay. As expected, it was found that DES sharply increased serum prolactin levels and enhanced cell proliferation in the anterior pituitary gland. DES also induced changes in parameters of vascularization. Simultaneous treatment of rats with SMS inhibited the DES-induced elevation of prolactin levels and pituitary cell proliferation. It also suppressed some but not all DES-induced changes in the anterior pituitary vascularization. These data suggest that the angio-inhibitory activity of SMS might be involved in its anti-tumor action on pituitary adenomas, but not as a sole or principal mechanism.

**Key words:** Somatostatin analogs, Octreotide, Prolactin, Cell proliferation, Vascularization, Anterior pituitary gland

## Introduction

The prolonged administration of high doses of estrogens is well known to induce the anterior pituitary hyperplasia and tumorigenesis in rodents (Zondek, 1936; Mietkiewski, 1959; Wiklund et al., 1981; Morgan et al., 1985). The estrogen-induced pituitary hyperplasia and tumor formation are accompanied by dramatic changes in the anterior lobe vasculature. These changes can be relevant for hyperplasia and tumorigenesis, since the newly formed arteries supply the gland with the systemic blood poor in dopamine (Elias and Weiner, 1984). The latter is a well known inhibitor not only of prolactin secretion, but of lactotroph proliferation as well. It has been recently shown in our laboratory that anti-angiogenic agents, fumagillin and TNP-470 exert a strong anti-tumor effect on diethylstilbestrol (DES)-induced pituitary tumors in rats (Stepien et al., 1996). One of the natural, endogenous substances possessing the anti-angiogenetic activity is somatostatin (Barrie et al., 1993). On the other hand, somatostatin analog has been found to exert the anti-tumor effect on rat transplantable pituitary tumor (De Quijada et al., 1983) and on growth hormone-secreting and non-functioning pituitary adenomas in human subjects (Plockinger et al., 1994). A question has arisen as to whether the anti-angiogenetic activities of somatostatin analogs are involved in their anti-tumor effects on pituitary adenomas. Since the dramatic changes of the anterior pituitary vasculature and lactotroph hyperplasia precede the adenoma formation in estrogen-treated rats, we have examined the effect of the octapeptide somatostatin analog octreotide on DES-induced vascular changes and cell proliferation in the anterior pituitary gland.

*Offprint requests to:* Prof. Dr. Marke Pawlikowski, Institute of Endocrinology Medical University of Lodz, Dr. Sterling Str. 3, 91-425 Lodz, Poland

## Materials and methods

### *Experimental protocol*

Four-week-old male Wistar rats, housed in a room with controlled illumination (light: darkness: 12:12) were used in the experiment. Silastic capsules containing 8-10 mg of diethylstilbestrol (DES, Sigma) each were implanted subcutaneously in the lumbar region. Such capsules were estimated to release 18-45 μg of DES daily (Wiklund et al., 1981). Control animals were

implanted with the empty capsules. Four weeks after the implantation of capsules, the DES-implanted rats were divided into two groups, 10 rats in each. The first group was injected s.c. twice daily with 25 μg of octreotide (Sandostatin Sandoz, SMS), for 10 days. The second group received injections of saline. Twenty-four hours after the last injection of SMS or saline, the animals were sacrificed. One hour before the sacrifice all the animals had received an intraperitoneal injection of bromodeoxyuridine (BrDU, Sigma). Blood and pituitaries were collected. The glands were fixed in Bouin-Holland fixative and embedded in paraffin using the routine procedure.

### *Prolactin assay*

Prolactin was assayed in blood serum using rat prolactin 125 J assay kit (Amersham, England).

### *Cell proliferation assay*

The paraffin sections (6 μm thick) were immunostained using a cell proliferation kit (Amersham International, England) to detect the incorporated BrDU. The labelling indices were calculated and expressed per 1000 cell nuclei. At least 3000 randomly scored nuclei were evaluated in each pituitary gland.

### *Quantitative evaluation of vasculature*

To quantify the vascular changes in the anterior pituitary gland, computer-assisted image analysis was applied. Paraffin sections (8 μm) were stained using the Seyle method with the modification of Poely et al. (1964) and then evaluated using Digital Image Analysis System IBAS 2000 (Kontron GmbH, Eching, Germany) attached to a black and white camera mounted on a light microscope. Fifty fields (0.195 $mm^2$) for every pituitary gland were randomly selected and analyzed. The microscopic image of a field seen in the camera was introduced into the computer using a green and then a red filter mounted in the optic system of the microscope. These procedures were used to distinguish vascular profiles, filled with red erythrocytes from the surrounding tissue (green stroma). Several parameters were assessed: 1) total vascular area-sum of the areas of all vascular profiles in the examined field, expressed as a percentage of pituitary tissue surface; 2) vessel density: number of vessel profiles in the examined field; 3) mean vessel profile diameter-mean of diameters of circles inscribed into all complete vascular profiles; and 4) mean vessel perimeter-mean vessel perimeter of all individual vessel profiles. The values of last three parameters concerned only vascular profiles totally included in the measuring frame and not crossing the border of the examined field. The first parameter informs about the blood supply and the others about the structure of the vascular network.

### *Statistical analysis of the data*

Statistical comparisons of data were carried out by one-way analysis of variance (ANOVA). The differences between means of prolactin concentrations and of BrDU labelling indices in the investigated groups were assessed by the Student's t test or the Mann-Whitney test. The differences in vascular parameters were assessed by Kolmogorov-Smirnov test. A level of $p<0.05$ was considered to be statistically significant.

## Results

### *Prolactin levels*

As expected, the implantation of DES resulted in a sharp increase in prolactin (PRL) levels (60.4±13.2 ng/ml in DES-implanted rats vs 6.8±1.7 ng/ml in controls). This increase was minimal and statistically insignificant in DES-implanted and SMS-treated animals (12.0±5.8

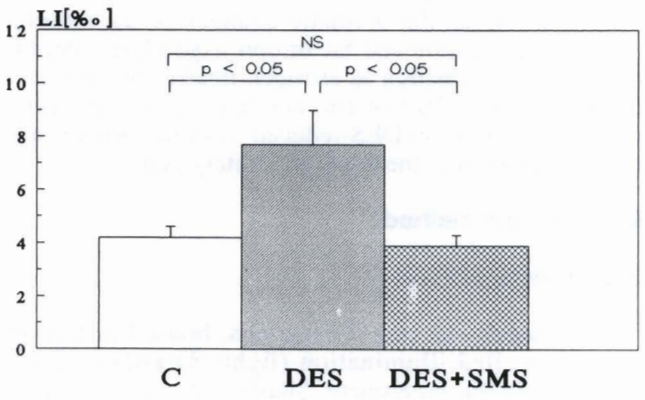



**Fig 1.** Effects of diethylstilbestrol (DES) and DES plus octreotide (DES+SMS) on bromodeoxyuridine (BrDU) labelling index (LI) of the anterior pituitary cell nuclei. Bars represent means + standard errors. C: controls.

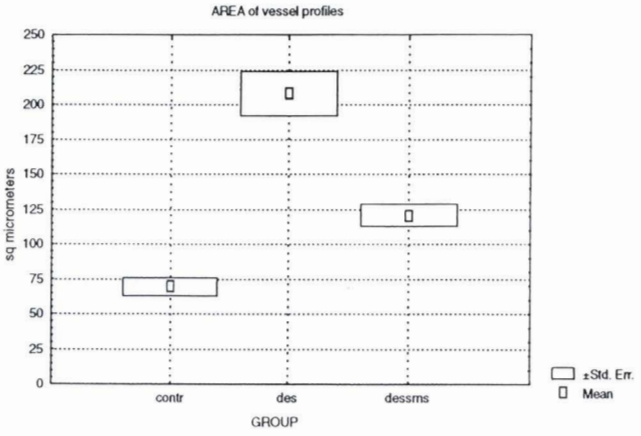



**Fig 2.** Effects of diethylstilbestrol (DES) and des plus octreotide (DES+SMS) on vessel area in the anterior pituitary tissue. Contr: controls. Results are means ± standard errors.

ng/ml)

*Cell proliferation*

Changes in BrDU labelling index are presented in Fig. 1. As expected, the labelling index was significantly higher in DES-implanted rats (7.7±1.3 %) in comparison to untreated controls (4.2±0.4 %). This increment did not occur in animals treated with SMS (3.9±0.4 %).

*Vascular parameters*

The implantation with DES was found to increase the vessel area, the mean vascular perimeter, mean vascular diameter and the vascular density (Figs. 2-5). The increases in the vessel area and vessel diameters were significantly attenuated by simultaneous treatment with SMS (Figs. 2, 5). However, SMS was unable to suppress the DES-induced changes in vascular density and vascular perimeters (Figs. 3, 4).

## Discussion

As expected, the implantation of rats with DES, a strong estrogenic compound, resulted in marked hyperprolactinaemia and hyperplasia of the anterior pituitary gland. As we have found in our earlier studies with the same experimental procedure, the hyperplasia involves mainly prolactin cells which compose 60-70% of pituitary cells in DES-treated rats (Pawlikowski et al., 1995, Stepien et al., 1996). The pituitaries of DES-treated cells exhibit the marked changes in their micro-vasculature. The increase in total vessel area, and in the mean vessel perimeter and diameter, observed in this study, is compatible with our previous observations (Pawlikowski et al., 1996; Stepien et al., 1996). However, in contrast to our earlier observations, we have also found increased vascular density in estrogen-treated rats. This discrepancy may depend on the different time point of investigation (6 weeks after the implantation in the present study vs 8 weeks in the earlier studies) as well as on the different strain of rats (Wistar vs Fisher 344). The treatment with the long acting somatostatin

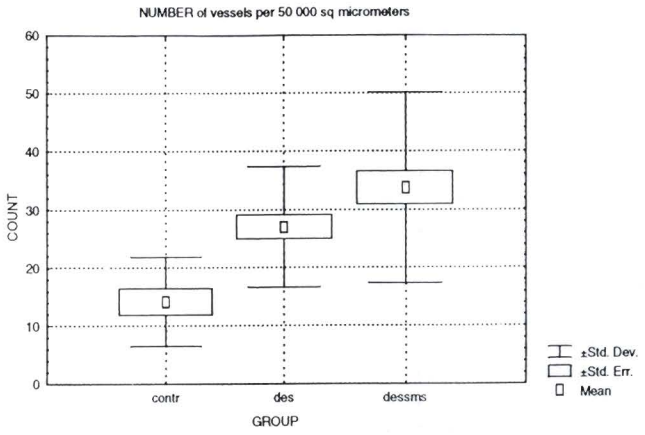



**Fig 3.** Effects of diethylstilbestrol (DES) and DES plus octreotide (DES+SMS) on the number of vascular profiles (vascular density) in the anterior pituitary tissue. Contr: controls. Results are means±standard errors and standard deviations.

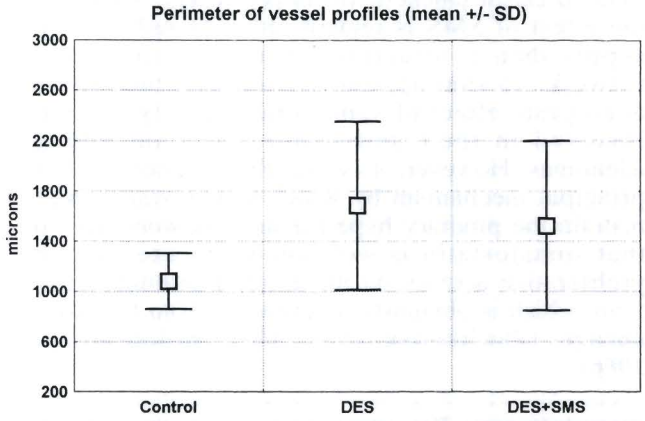
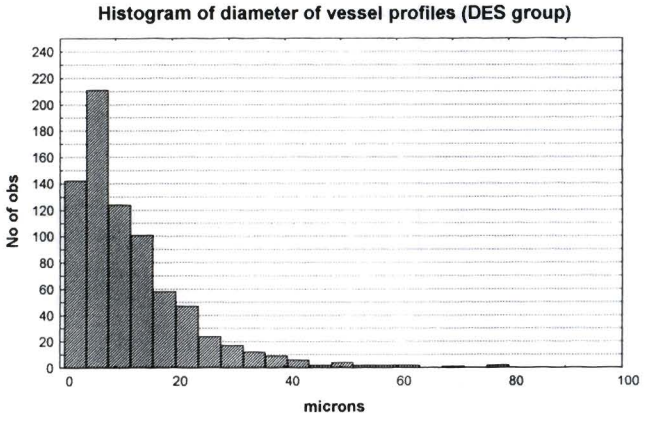



**Fig 4.** Effects of diethylstilbestrol (DES) and DES plus octreotide on perimeter of vessel profiles. Results are means ± standard deviations.



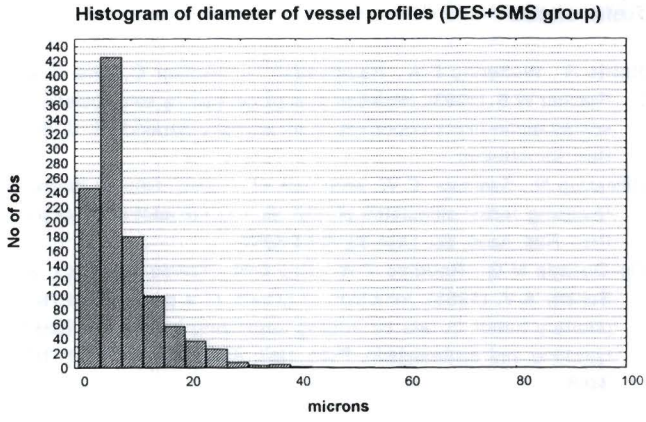




**Fig 5.** Distributive series of vessel diameters in the anterior pituitary tissue in diethylstilbestrol (DES) and DES plus octreotide (DES+SMS) treated-rats.

analog, octreotide (SMS) prevented almost all the changes evoked by estrogen administration. The inhibition of hyperprolactinaemia is compatible with the finding of Lee and Shin (1996) who observed that somatostatin inhibited PRL synthesis *in vitro* in pituitary cells derived from estrogen-primed rats but not in cells derived from normal rats. Recently, Zhang et al. (1996) have found that estradiol stimulated the expression of somatostatin receptor subtypes SST2 and SST3; the latter being thought to be related to PRL inhibition. SMS was also effective in inhibiting the estrogen-induced cell proliferation. This finding is compatible with the data of Schussler et al. (1994) who observed the same effect using another long acting somatostatin analog BIM 23014 (Somatuline). SMS treatment also inhibited or attenuated the DES-induced vascular changes. Its effect is roughly similar to those exerted by such angiogenesis inhibitors as fumagillin and TNP 470 (Stepien et al., 1996). Unexpectedly, the inhibitory effect of SMS did not involve the vascular density. This parameter is even slightly enhanced in SMS-treated rats, which suggests that the reduction in parenchyma volume under the influence of SMS exceeds the eventual involution of the vascular network. SMS also failed to suppress the DES-induced enhancement in vessel perimeters. It seems that the effect of SMS is related rather to reduced blood supply than to structural changes in the vascular network. To sum up, our data suggest that the anti-angiogenic effect of somatostatin analogs might be involved in their anti-tumor effect on pituitary adenomas. However, it is probably neither a sole nor principal mechanism by which somatostatin analogs restrain the pituitary hyperplasia. It is worth recalling that somatostatin is well known to exert an anti-proliferative activity on the anterior pituitary cells *in vitro*, which is obviously independent from the vascular changes (Pawlikowski et al., 1978, Billestrup et al., 1986).

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