The effect of mesulergine on prolactin secretion and anterior pituitary cells morphology in diethylstilboestrol-treated female Wistar rats

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Summary. Mesulergine (Cu32-085) is an active semisynthetic ergot alkaloid with unusual biphasic antagonistic-agonistic effect on dopamine (DA) turnover in the rat striatum. The present study has been made to elucidate the influence of the long-term treatment of this drug on prolactin secretion and prolactin cells morphology in the female Wistar rats with experimentally-induced hyperprolactinemia. Additionally, the effect of this drug was compared with bromocriptine and pergolide activity, applied in the same experimental conditions. It has been shown that prolonged mesulergine treatment attenuated the stimulatory effect of stilboestrol on prolactin secretion *in vivo*. It also decreased mean prolactin cells density, above all cells and lactotroph mitotic indexes, estimated in immunohistochemically-stained slides. However, antiproliferative activity of Cu 32-085 was weaker, when compared with bromocriptine and pergolide.

Key words: Mesulergine, Prolactin secretion, Immunohistochemistry, Antiproliferative action

Introduction

It is well established, that bromocriptine and other D_2-dopamine agonists, like pergolide, lisuride and cabergoline not only inhibit prolactin secretion, but also exert an antiproliferative action on pituitary prolactin cells. The antiprolactin action of semisynthetic ergot alkaloid derivatives is well documented in researches performed on experimental animals (Dannies and Rudnick, 1980; Maurer, 1981; Eljarmark et al., 1985a) and humans, both *in vivo* (Kleinberg et al., 1983; Barrow et al., 1984; Bassetti et al., 1984) and *in vitro* (Duffy et al., 1988). Recent morphological reports have demonstrated dramatic reduction in the size of large prolactin-producing adenomas after bromocriptine and pergolide treatment, suggesting that it is a result of the reduction in tumour cell volumes, rather than a reduction in tumour cell numbers (Rengachary et al., 1982; Tindall et al., 1982; Anniko and Wersall, 1983; Horowitz et al., 1983; Kleinberg et al., 1983). Also, a decreased mitotic rate, pycnosis and disappearance of nuclei in the rat tumour tissue were observed after treatment with natural and semisynthetic ergot alkaloid derivatives (Quadri et al., 1972; Mac Leod and Lehmeyer, 1973).

Mesulergine (Cu 32-085) was developed in the late seventies at Sandoz laboratory. It belongs to 8-α-aminoergoline derivatives and was recently characterized as a drug with low antiprolactin potency, when compared with bromocriptine and pergolide. This compound, unlike other dopamine agonists, exhibits an unusual pharmacological profile with a time-dependent, biphasic antagonistic-agonistic effect on DA turnover in the rat striatum (Flückiger et al., 1979, 1983; Enz, 1981). In our previous study (Pisarek et al., 1991) we demonstrated that simple injection of Cu 32-085 in rats attenuated the stimulatory effect of diethylstilboestrol (DES) treatment on serum prolactin level in a time- and dose-dependent fashion. Since no data are yet available on the influence of mesulergine on pituitary cells morphology, the present study was designated to elucidate the effect of long-term treatment with this drug on the lactoroph morphology and prolactin secretion in the female Wistar rat with experimentally-induced hyperprolactinemia. The antiprolactin potency of mesulergine was compared with bromocriptine and pergolide action, applied in the same experimental conditions.

Materials and methods

Animals

Female Wistar rats, weighing 130-140 g and maintained on tap water and food *ad libitum* in a light- and temperature-controlled room, were used in the experiments. The animals underwent chronic oestrogen treatment, using the following regime: capsules were prepared from 5 mm-long Silastic tubing, 1.57 mm internal diameter, 2.4 mm external diameter (Dow Corning Co., Midland, Mich., USA), filled with a saturated solution of diethylstilboestrol (DES; Sigma Chemical Co., St. Louis, Mo., USA) in 95% ethanol. After evaporation of the alcohol, the capsules, containing 8-10 mg DES each, were sealed with Silastic medical adhesive (Dow Corning Co.), protected from light and stored at 4° C. These capsules were then implanted subcutaneously under ether anaesthesia in the lumbar region of each rat. Such implants were estimated to release 18-45 µg/day of DES (Wiklund et al., 1981) and to induce massive hyperplasia of the prolactin-secreting pituitary cells in the used strain of rats. Empty Silastic capsules were implanted in intact rats treated as control.

The substances to be tested:

1. Mesulergine - (Cu 32-085) - N,N-dimethylsulphamide, N'-1,6-diethyloergoline-8-α-yl), Sandoz AG., Product Limited, Basel, Switzerland.
2. Bromocriptine - (CB 154) - 2-bromo-α-ergocriptine, methane sulphonate, Sandoz AG., Product Limited, Basel, Switzerland. Both substances were given free of charge from Sandoz Laboratory, thanks to Prof. E. Flückiger.
3. Pergolide (LY 127809) 8-ß-8-(methylothiomethylo)-6-propyloergoline, given free of charge from the Lilly Laboratories, Eli Lilly and Co., Indianapolis, USA.

Schedule of experiments:

The animals were divided into 5 experimental groups, 8 rats in each, in the following manner:
Group I - contained rats with empty implanted Silastic tubes (without DES).
Group II - contained animals with implanted Silastic tubes with DES.
The animals in these groups received subcutaneous injections of 0.2 ml 1% lactic acid, which is the diluent for tested ergot alkaloids. The remaining groups consisted of rats with implanted DES tubes, which received as follows:
Group III - mesulergine in a dose of 3.0 mg/kg of body weight/24 h in 0.2 ml volume.
Group IV - bromocriptine in a dose of 3.0 mg/kg of body weight/24 h in 0.2 ml volume.
Group V - pergolide in a dose of 3.0 mg/kg of body weight/24 h in 0.2 ml volume.

Injections were made twice per 24 hours at 12 hour intervals, for 10 days. 2 hours after the last injection all animals received vincristine (Vincristine, Gedeon Richter, Budapest, Hungary) intraperitoneally at a dose of 1 mg/kg b.w. to stop cell divisions in metaphase stage. 6 hours after vincristine injection all rats were decapitated, the blood and pituitaries from them being collected. After centrifugation (1800 g x 15 min), the plasma was stored at -20° C until it was analyzed for PRL. Rat pituitaries were fixed in Bouin's fluid and prepared for further morphological study.

Prolactin cells immunohistochemistry

For all the 5 µm paraffin-embedded pituitary slices studied, the classical Sternberger sandwich PAP method (Sternberger et al., 1979) was applied. After removing endogenous peroxidase and nonspecific binding the sections were incubated with rabbit anti-rPRL antiserum (1:100 dilution; UCB Bioproduct, Belgium). Then slices were incubated with goat anti-rabbit immunoglobulin serum (1:20; 65-036 Miles Lab, USA) and afterwards with PAP complex (1:100; UCB, Bioproduct, Belgium). All the incubations were performed for 1 h at 37° C in a damp atmosphere. AEC (Aminoethylcarbazol, Dakopatts, Denmark) was used as a chromogen in all the slices studied. Sections incubated with PBS buffer instead of the primary antibody, served as a negative control.

Estimation of density of prolactin cells

Mean density of prolactin cells in microscopic slices was measured by using a 10-point scale microscopic lense, according to Mc Nicol et al. (1988) with our slight modification. The point falling on the nuclei of cells with immunopositive cytoplasm were counted as positive. The number of prolactin cells per 1000 anterior pituitary cells was determined. Mean value from 8 pituitaries in each measured group, was expressed as percentage.

Estimation of mitotic indexes

Overall mitotic index was calculated as the number of mitoses (at metaphase stage) per 1000 anterior pituitary cells. 3500 cells were counted in one slice. Prolactin cells mitotic index was calculated as the number of cell divisions at metaphase in immunopositive lactotrophs per 1000 prolactin cells. Minimum 1500 cells were counted in each slice.

Hormone radioimmunoassay

Rat prolactin plasma level was determined by radioimmunoassay kit produced by DRG (r-PRL, RE 49301, DRG International Inc. Mountainside, N.Y., USA), using double antibody method. Sensitivity in this method was 0.5 ng/ml. Inter- and intra-assay variance was 11% and 7% respectively.

Statistic analysis

All data were statistically evaluated by one-way analysis of variance and the Student's t-test. Differences with a p value < 0.05 were considered as significant. All mean values are expressed $\pm$ standard error of the mean.

Results

a) Morphological studies

The results of experiments concerning mesulergine, bromocriptine and pergolide treatment on the pituitary prolactin cells density and mitotic indexes in immunohistochemically-stained slides are shown in Fig. 1 and Table 1 (see also Figs. 2a-f). The highest density of prolactin cells occurred in hyperprolactinemic rats which were treated for 10 days with alkaloid diluent (Gr. II). Prolactin cells percentage in tested slides was lowest in the animals which received pergolide (Gr. V). Prolactin cells quantity after bromocriptine (Gr. IV) and mesulergine (Gr. III) treatment was significantly lower as well in comparison with the animals which received the ergotderivative diluent. However, bromocriptine effects were stronger than those of mesulergine ($p < 0.001$). Overall pituitary cells mitotic index in ovariectomized rats after diethylstilboestrol treatment (Gr. II) was significantly higher in comparison with ovariectomized (Gr. I) rats ($4.30 \pm 0.48\%$ versus $0.52 \pm 0.08\%$). Index values decreased upon the influence of mesulergine, bromocriptine and pergolide injections and were $3.12 \pm 0.48\%$, $2.44 \pm 0.65\%$ and $1.36 \pm 0.31\%$ respectively. There were no statistically significant differences in the mean values of mitotic indexes among mesulergine- and bromocriptine-
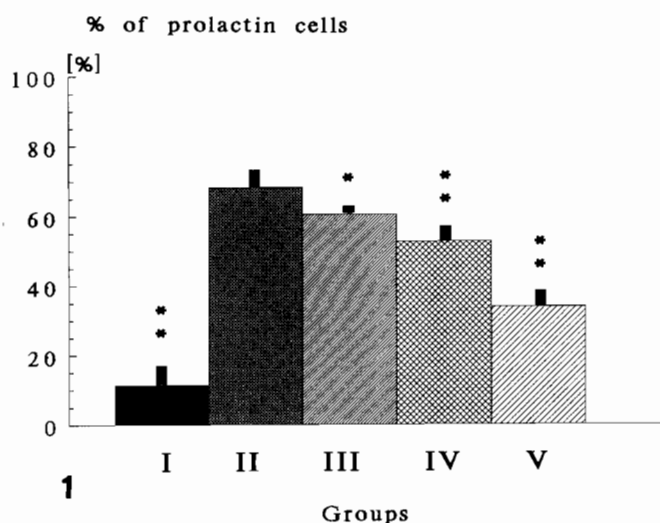
Fig. 1. Prolactin cells quantity in 1000 anterior pituitary cells after 10 days of different dopamine D_2-receptor agonists treatment. The mean values expressed in percentage (p tested vs Gr. II; * p < 0.05, ** p < 0.001).
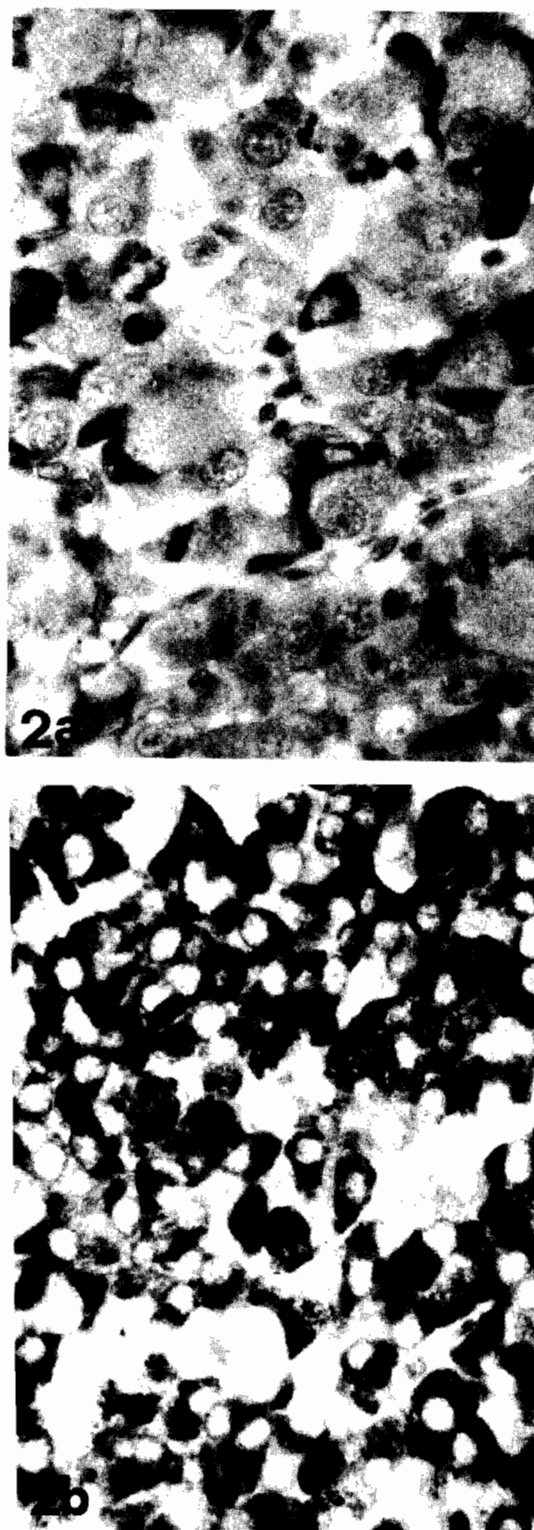
Fig. 2. Immunohistochemical localization of prolactin cells in the rat anterior pituitary gland. **a)** bilateral ovariectomy, **b)** DES treated rats, **c)** mesulergine treatment, **d)** bromocriptine treatment, **e)** pergolide treatment, **f)** mitosis in immunopositive prolactin cells. Immunoperoxidase technique for demonstration of r-PRL. × 630
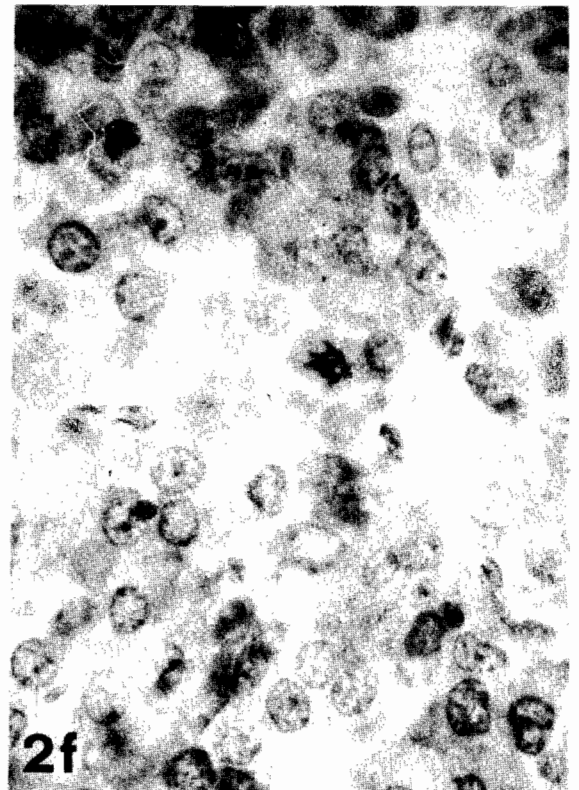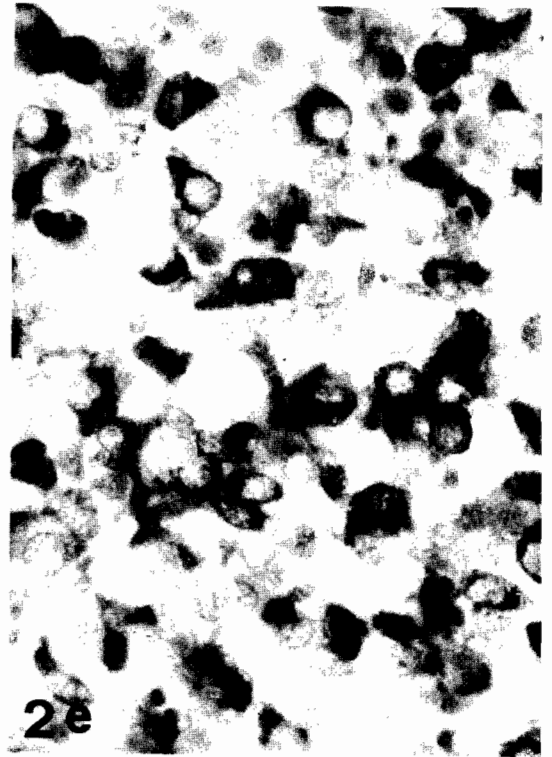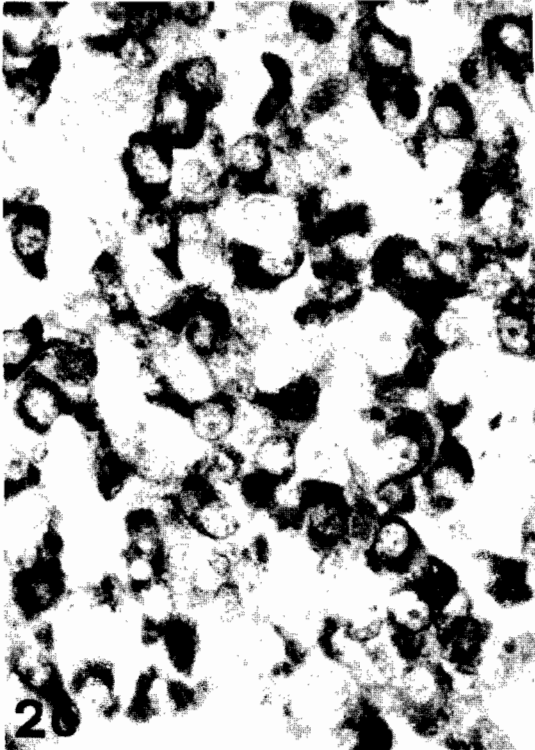
treated groups (p > 0.05). Therefore, a significant difference was observed in the mean mitotic index values between mesulergine- and pergolide-treated groups (p < 0.001).

Prolactin cell mitotic index was 2.76 ± 0.16% in group II and was significantly higher than in control

(Gr. I). All drugs decreased this index value to the level 2.34 ± 0.20% for mesulergine, 1.76 ± 0.43% for bromocriptine and 0.98 ± 0.13% for pergolide.

b) Secretory studies

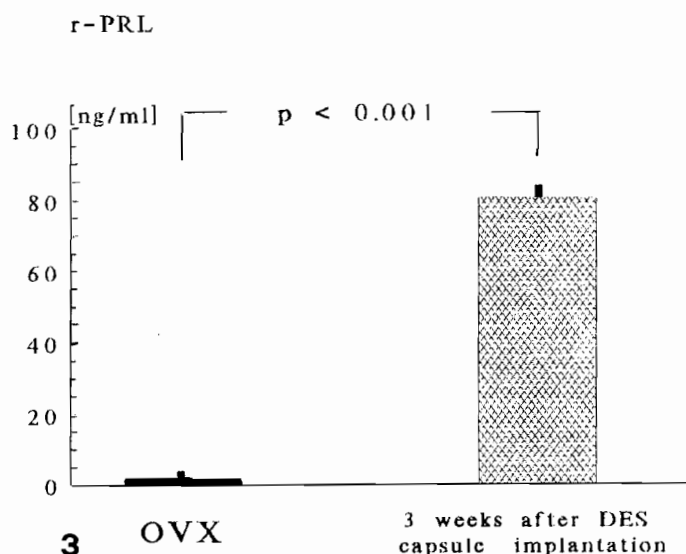The mean serum prolactin level in female Wistar

r‑PRL

Fig. 3. Prolactin level in female Wistar rats blood serum after bilateral ovariectomy (OVX) and 3 weeks after DES capsules implantation (OVX + DES).

r‑PRL

Fig. 4. Female Wistar rats blood serum prolactin level (r‑PRL) after 10 days of mesulergine, bromocriptine and pergolide treatment in hyperprolactinemic conditions (p tested vs Gr. II).

Table 1. Mitotic index values (overall and prolactin cells index) after 10 days of mesulergine, bromocriptine and pergolide treatment at a dose of 3.0 mg/kg b.w. (p tested vs Gr. II).

	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V
overall mitotic index	0.52 ±0.08	4.30 ±0.48	3.12 ± 0.48	2.44 ± 0.65	1.36 ± 0.31
	$p < 0.001$		$p < 0.01$	$p < 0.001$	$p < 0.001$
prolactin cells mitotic index	0.34 ± 0.16	2.76 ± 0.16	2.34 ± 0.20	1.76 ± 0.43	0.98 ± 0.13
	$p < 0.001$		$p < 0.001$	$p < 0.001$	$p < 0.001$

rats after bilateral ovariectomy with implanted empty capsules (without DES) was 1.67 ± 0.23 ng/ml. As expected, 3 weeks after implantation of tubes containing DES, prolactin level increased significantly to the value of 80.83 ± 3.80 ng/ml ($p < 0.001$), (Fig. 3). 10 days after mesulergine, bromocriptine and pergolide treatment prolactin level decreased. The most potent drug was pergolide, which decreased prolactin level to 4.18 ± 1.01 ng/ml, whereas bromocriptine lowered prolactin concentration to 8.46 ± 5.77 ng/ml. Mesulergine exerted the weakest inhibitory effect and decreased prolactin level to 14.71 ± 4.44 ng/ml. Prolactin concentration in individual groups, receiving tested drugs, statistically differed between each other (mesulergine vs bromocriptine: $p < 0.05$; mesulergine vs pergolide: $p < 0.001$), (Fig. 4).

Discussion

It has already been shown that several natural and semisynthetic ergot alkaloids strongly inhibit prolactin secretion both *in vivo* and *in vitro* (Mac Leod and Lehmeyer, 1973; Grossman et al., 1980; Barrow et al., 1984). Also demonstrated has been their inhibitory influence on the anterior pituitary DNA synthesis and mitotic activity of prolactin cells (Davies et al., 1974; Lloyd et al., 1975; Pawlikowski and Stepien, 1979). Pharmacological action of these drugs is connected with their agonistic influence on the D_2‑dopamine receptors, located both on hypothalamic tuberoinfundibular (TIDA) neurons (Flückiger and Vigouret, 1981; Gudelsky, 1981) and on the surface of pituitary lactotrophs (Ben‑Jonathan, 1985; Eljarmark et al., 1985b).

Recently, several neuropharmacological experiments have been done with a relatively new diethyloergoline derivative - mesulergine. They proved that Cu 32‑085 decreased prolactin synthesis and release from anterior pituitary cells (Flückiger et al., 1979; Lamberts et al., 1984; Marko, 1984). In our previous studies (Pisarek et al., 1991) we also showed that mesulergine at a concentration range between 10^{-5} 10^{-7} M inhibited prolactin secretion from cultured rat pituitary cells to the medium during 180 min incubation in a dose‑dependent manner. It was also shown that chronic mesulergine treatment (in a dose of 3.0 mg/kg b.w. injected s.c. for 10 days in hyperprolactinemic conditions) induced a significant decrease in the number of dopamine D_2‑binding sites, without any changes in D_2‑receptor affinity. This suggests that antiprolactin activity of this drug *in vivo* and *in vitro* is probably associated with its agonistic effect to D_2‑dopamine receptors (Pisarek et al., 1990). In the present study we have found that mesulergine abolishes the stimulatory effect of DES on prolactin secretion in ovariectomized rats. This inhibitory effect is less potent (79.7%), when compared to bromocriptine (88.3%) and pergolide (94.2%).

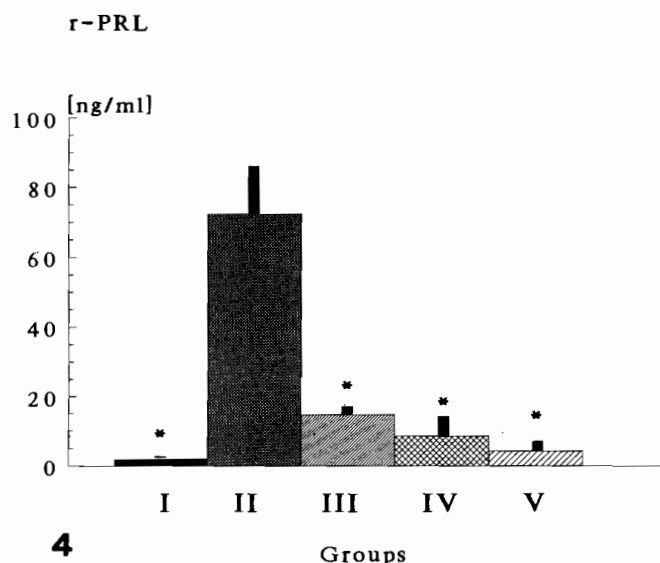Less attention was paid to determining the

morphological changes after long-term mesulergine treatment. The immunohistochemical investigations clearly show the differences in pituitary prolactin cell structures after ovariectomy, DES implantation and ergot alkaloid treatment. A dramatic fall was observed in prolactin cells density after ovariectomy, and its remarkable rise as a consequence of long-term DES action. These observations are in agreement with previous investigations, where already 8 days after DES capsules implantation, prolactin cells made up about 50% of the anterior pituitary cell population (Phelps and Hymer, 1983). Additionally it has been suggested that decreased dopamine synthesis in the hypothalamic TIDA neurons, induced by long-term DES treatment, is responsible for stimulation of the proliferation (Sarkar et al., 1984) and density of pituitary lactotrophs (Cronin et al., 1982). Our present immunohistochemical studies show that ovariectomy causes a significant decrease in quantity and size of lactotrophs. They contained a small prolactin-positive granulation which occupied a relatively small cytoplasmic area. Many castrative, prolactin-negative cells were seen, whose optically empty cytoplasm occupied a considerable percentage of the cell area. Mitotic index in this group was also the lowest. After DES implantation the density of prolactin cells and number of prolactin-positive granules in the cytoplasm rose remarkably. The mitotic index in this group was also increased. These observations are in agreement with previous results, which indicated a strong proliferogenic oestrogen action on the anterior pituitary gland (Lloyd et al., 1975; Pérez et al., 1986). The long-term treatment with mesulergine, bromocriptine and pergolide, also induced characteristic changes in lactotrophs morphology. The intensity of these changes was due to the antiprolactin secretory activity of the tested compound. The greatest decline in density of prolactin cells appeared after pergolide treatment (33.9% all pituitary cells). Bromocriptine and mesulergine decreased this value to 52.6% and 60.4% respectively. The lactotrophs mitotic activity was also remarkably inhibited by all drugs tested, but the antiproliferative effect of mesulergine was weaker, when compared with bromocriptine and pergolide. The mechanism of antiproliferative action of ergot alkaloids on the postestrogenic pituitary growth and prolactinoma development still remains unclear. Some authors (Barrow et al., 1984; Duffy et al., 1988) suggest that the decrease in size of the prolactin adenoma is a consequence of reduction of the following: cytoplasm volume, endoplasmatic reticulum and Golgi apparatus, rather than the fall in the total number of prolactin cells. On the other hand, Mc Comb et al. (1986) suggest that antiproliferative action of bromocriptine is connected with its direct inhibitory influence on the pituitary cells mitotic activity. The significant changes in the values of cell organelle surface under the influence of the CB-154 were not described by them. In the light of our studies it is possible to suggest that antiprolactin action of mesulergine is related both to its inhibitory effect on prolactin secretion and the decreased mitotic rate of pituitary lactotrophs.

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