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The Effect of Bromocriptine on the Lactotroph Cells in Two Inbred Strains of Mice C_3H/Sy and AKR/Sy

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Abstract—*This paper deals with the effect of bromocriptine on the morphology and the number of lactotrophs in two inbred strains of mice, one with a high and the other with a low mammary cancer incidence.*

Bromocriptine decreases the number of lactotrophs in C3H mice while their number in AKR is not affected. These observations suggest that bromocriptine affects the two strains in a different way, probably due to their different prolactin levels in the pituitary.

The above treatment resulted also in a shrinking of the RER and a reduction in size of prolactin granules, probably due to a decline in the secretory activity of lactotrophs.

Of special interest was the appearance of a 'new' cell type with mixed characteristics of somatotrophs and lactotrophs.

These findings, along with an increase in the number of chromophobes, suggest that bromocriptine can induce cell regression. Probably the 'new' cell is an intermediate form between chromophobes and lactotrophs and somatotrophs.

INTRODUCTION

BROMOCRIPTINE belongs to the ergot alkaloids. Its main action is the inhibition of prolactin secretion from the lactotrophs of the adenohypophysis, lowering the prolactin levels in the blood. Bromocriptine has no effect on other pituitary hormones with the exception of growth hormone [1].

Prolactin is believed to play a key role in mammary carcinogenesis [2]. Thus, in this paper we studied the effect of bromocriptine on the morphology and the number of lactotrophs of the adenohypophysis in two inbred strains of mice, one with a high and the other with a low mammary cancer incidence, C3H/Sy and AKR/Sy, respectively.

MATERIALS AND METHODS

A total of 50 virgin female C3H/Sy and AKR/Sy inbred mice, about 4 months old, were used. The animals were obtained from the Experimental Department of the Theagenion Cancer Institute. At the beginning of the experiment the animals were weighed and housed in individual cages.

The bromocriptine suspension was prepared by dissolving the drug initially in a minimal amount of ethanol and diluting it to volume with a 0.89% NaCl solution. Ethanol consisted of 2.5% or less of the final suspension.

Three groups of mice from each strain were studied. The first group, which was the experimental one, consisted of virgin female mice (10 mice from each strain). These animals were given 0.1 mg/0.1 ml bromocriptine suspension intraperitoneally, daily for 4 weeks.

The second group consisted of virgin female mice (10 mice from each strain). This group received daily only the vehicle for 4 weeks (control group).

The third group (5 mice from each strain) consisted of virgin female mice which did not receive any treatment at all (control group).

The animals at the end of the experiment were sacrificed by ether during the estrus phase and the pituitaries were excised with the sella turcica and the diaphragma sellae. They were fixed *en bloc* in 2.5% glutaraldehyde in Sörrensen medium (pH 7.2). After hardening, the pituitary was carefully dissected from the diaphragma sellae and the sella turcica, and divided in four blocks of tissue. These were postfixed in 1% osmium tetroxide, dehydrated through graded alcohols and embedded in Epon.

Thin sections on copper grids were stained with uranyl acetate, poststained with lead citrate and

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examined in an Elmiskop I electron microscope.

As stated before, four blocks of tissue were obtained from each hypophysis. From each block, four 200 mesh copper grids were filled with sections. At least 16 such grids from each hypophysis were examined. The sections of each grid were cut at different levels. This way we tried to have a representative population of cells in each one. In each grid we counted only those sections that completely covered a grid hole. We counted the cells of at least 80 grid holes, all in all abut 3000 cells from each hypophysis.

This way, although we could not calculate the absolute number of lactotroph cells, we could evaluate their mean variation in each hypophysis.

RESULTS

While the number of lactotroph cells in AKR mice remained constant, there was, by comparison, a 25% decrease of these cells in C3H mice. It must be noted, however, that the lactotrophs in the C3H adenohypophysis are twice as numerous as in the AKR mice.

In parallel, the administration of bromocriptine caused some morphological changes in the cells of the adenohypophysis and especially the lactotrophs. Namely it reduced the size of secretory granules of lactotrophs in the experimental groups (Figs. 1, 3) as compared to the control mice (Figs. 2, 4). This reduction was more pronounced in C3H than in AKR mice (Figs. 1, 3). Additionally, the normally dilated rough endoplasmic reticulum in control mice (Figs. 2, 4) underwent shrinkage in both experimental groups after bromocriptine treatment (Figs. 1, 3).

Of special interest was the appearance of a 'new' cell type with mixed characteristics of somatotrophs and lactotrophs, especially concerning the shape of their secretory granules. The frequency of appearance of this 'new' cell type was the same in both strains of mice (Figs. 5, 6).

Finally, an increase in the number of chromophobe cells was observed in both strains.

There were no morphological differences among the animals of the control groups: those treated with the vehicle and the untreated group.

DISCUSSION

From the literature it is known that the administration of bromocriptine in mice inhibits the prolactin secretion from the lactotrophs of the adenohyphophysis [2], reduces the incidence of mammary tumors [3] and inhibits the growth of tumors at the first stages of their development [4]. However, it is ineffective on tumors which are not prolactin dependent [5].

In this paper it was found that bromocriptine causes a decrease in the number of the lactotrophs in the adenohypophysis of C3H mice. This inbred strain has a high incidence (99%) of spontaneous mammary cancer [6]. On the other hand we found that it does not affect the number of these cells in AKR mice, which according to Festing [7] have a low incidence of spontaneous mammary cancer, less than 1%.

It is widely accepted that there are some hormonal differences between these two strains of mice [8], the main difference being the amount of prolactin in their pituitaries. So, pituitary concentrations of prolactin appear to be generally higher in strains with a high incidence of mammary tumors, such as C3H, than in AKR mice, a strain in which mammary tumor incidence is very low [9].

Lloyd *et al.* [10] administered both estrogens and bromocriptine in rats and they found under the influence of these drugs lactotrophs achieved a high intracellular concentration of prolactin. On the other hand, they found that these intracellular concentrations of prolactin reduce mitotic activity in lactotrophs. These findings led them to support the view that the secretory behavior influences the mitotic activity in lactotrophs and that an intracellular negative feedback may exist between intracellular concentrations of the hormone and mitotic rate.

It appears, therefore, that due to the normally high prolactin levels in C3H mice on the one hand and to the blocking of prolactin release by bromocriptine on the other, there is an acute increase in pituitary prolactin content [2]. The negative feedback of Lloyd *et al.* [10] takes over and the number of lactotrophs is subsequently decreased. On the contrary, due to the normally lower prolactin levels in AKR mice in the one hand and because bromocriptine blocks only increased PRL secretion on the other, the negative feedback of Lloyd *et al.* [10] does not exist and the number of lactotrophs is not affected in this strain.

Moreover, we observed some morphological changes in lactotrophs in both treated strains of mice at the ultrastructural level. We noticed a shrinking of the rough endoplasmic reticulum and a reduction in the size of prolactin granules. This was more noted in C3H mice. It is thus possible to conclude that the lactotrophs of C3H mice were more sensitive to the effects of bromocriptine treatment than those of AKR mice.

Suoto and Dumm [11] report that a more developed rough endoplasmic reticulum means a higher synthesis of hormone. So, perhaps the above findings are morphological consequences of reduced hormonal synthesis. These findings are in accordance with those of McComb *et al.* [12] who report that the effect of bromocriptine may well be twofold: to inhibit both the synthesis and the release of the hormone, and contrary to those of Antakly *et al.*

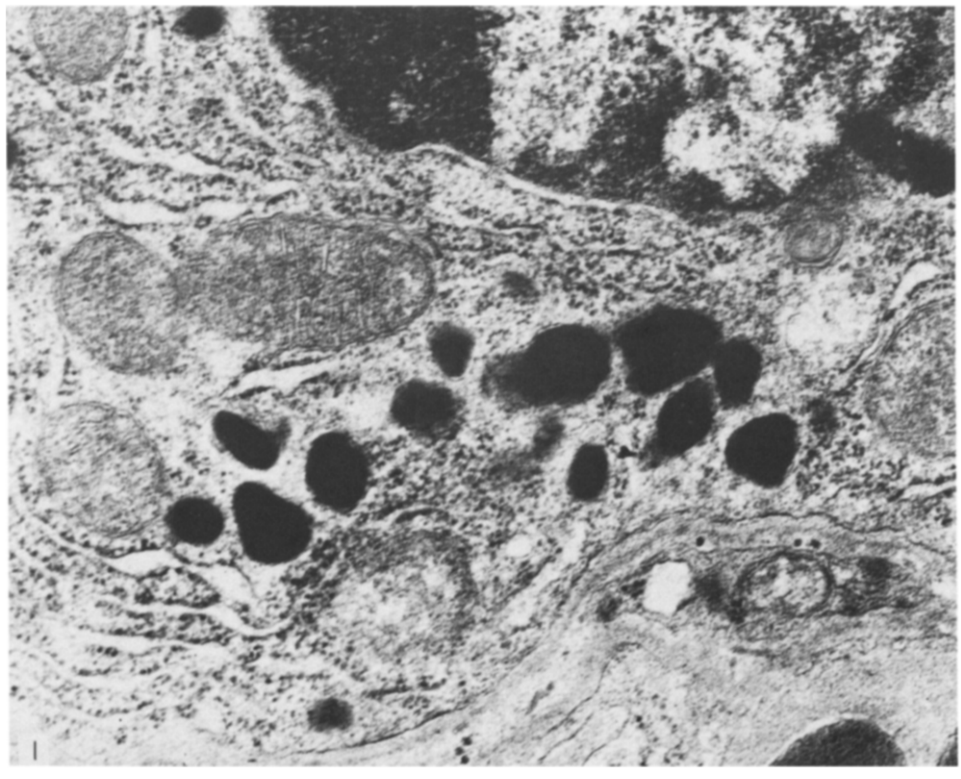

Fig. 1. Adenohypophysis of virgin female C3H mouse after a 4-week treatment with bromocriptine. Part of a lactotroph cell with smaller prolactin granules and less developed granular endoplasmic reticulum than in controls (compare with Fig. 2). ×24,000. (In all figures, bar = 1 μm, G = growth hormone granules, P = prolactin granules.)

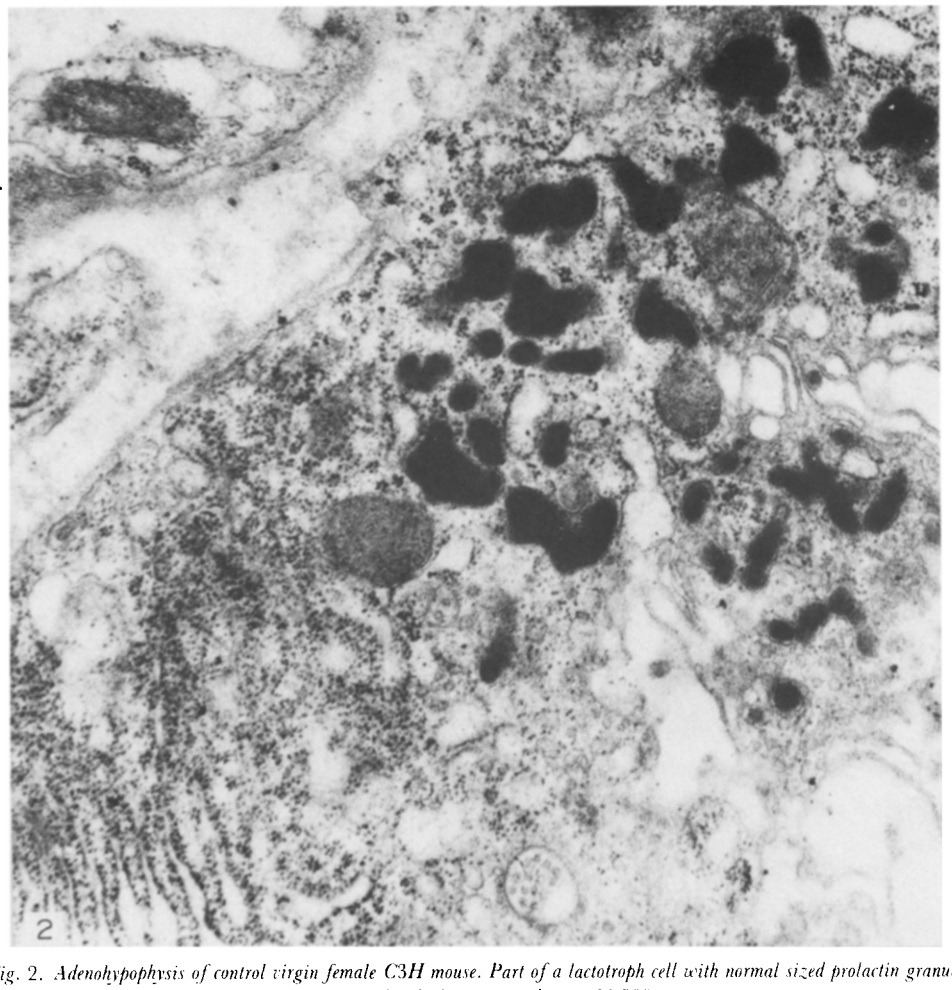

Fig. 2. Adenohypophysis of control virgin female C3H mouse. Part of a lactotroph cell with normal sized prolactin granules and normal endoplasmic reticulum. ×24,000.

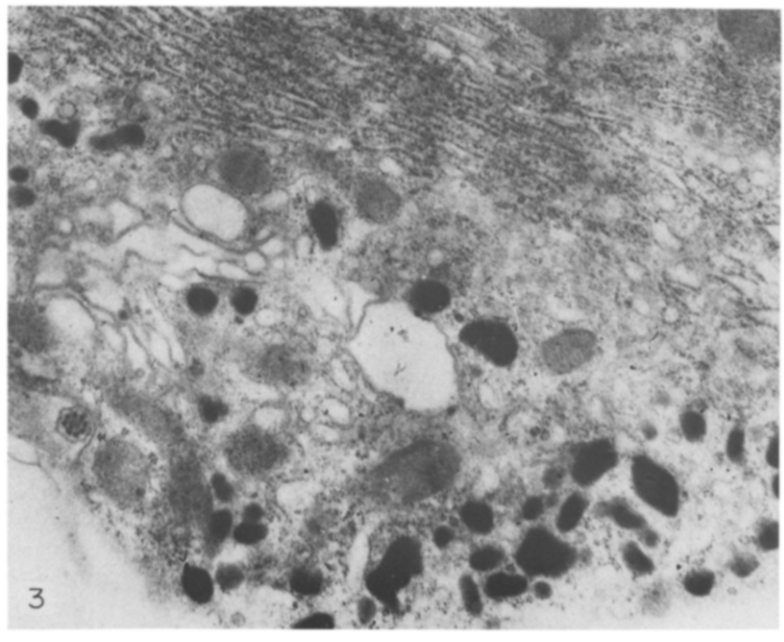

Fig. 3. Adenohypophysis of virgin female AKR mouse after a 4-week treatment with bromocriptine. Part of a lactotroph cell with smaller prolactin granules and less developed granular endoplasmic reticulum than in controls (compare with Fig. 4). ×24,000.

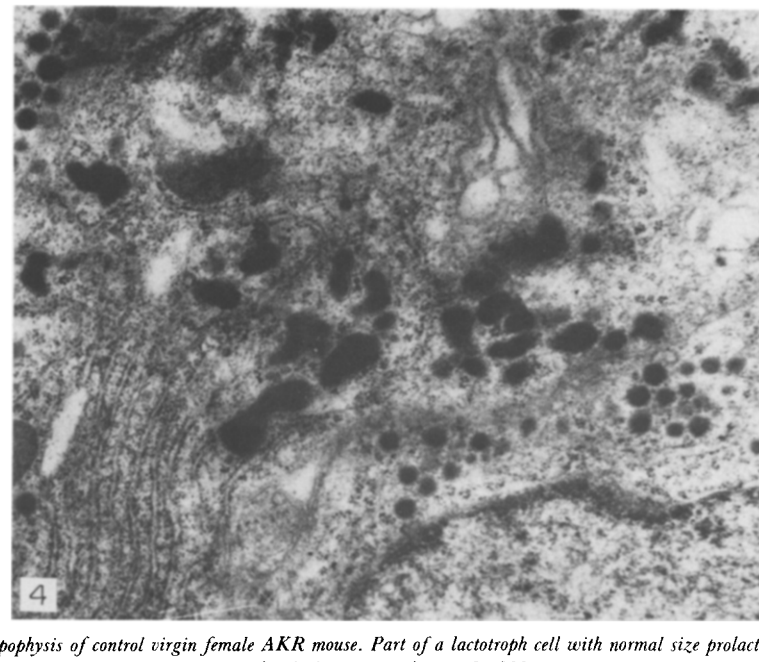

Fig. 4. Adenohypophysis of control virgin female AKR mouse. Part of a lactotroph cell with normal size prolactin granules and normal endoplasmic reticulum. × 24,000.

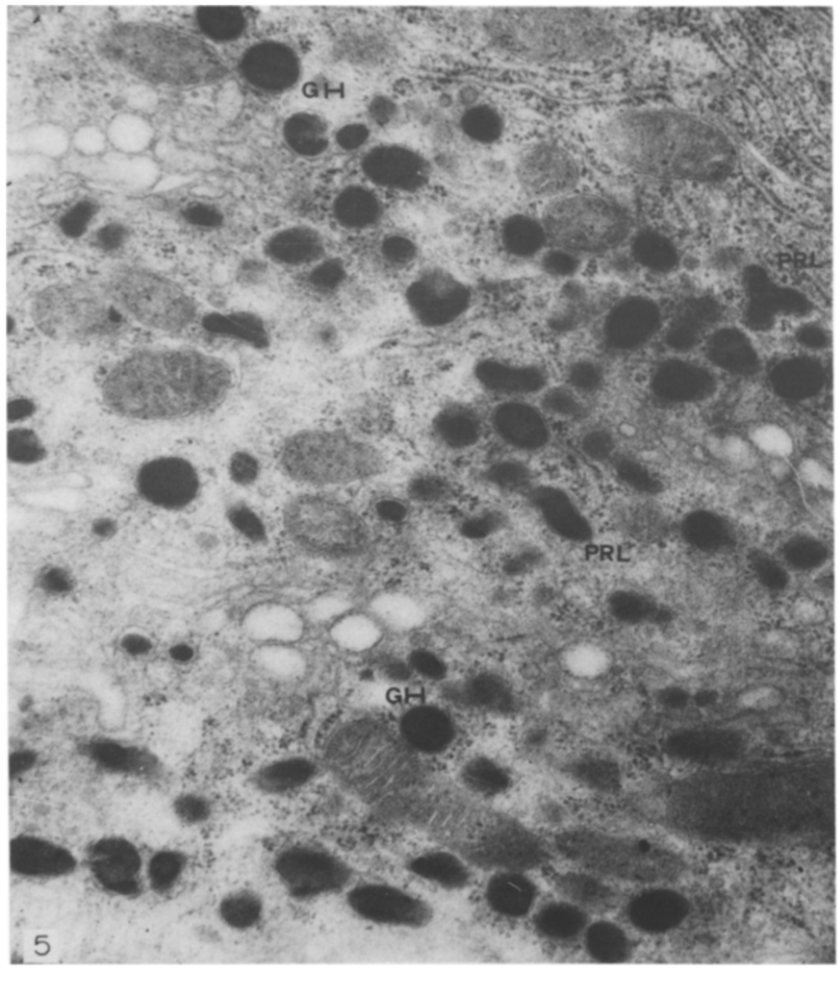

Fig. 5. Adenohypophysis of virgin female C3H mouse after a 4-week treatment with bromocriptine. Part of a 'new' cell type with mixed characteristics of somatotroph and lactotroph cells. ×24,000.

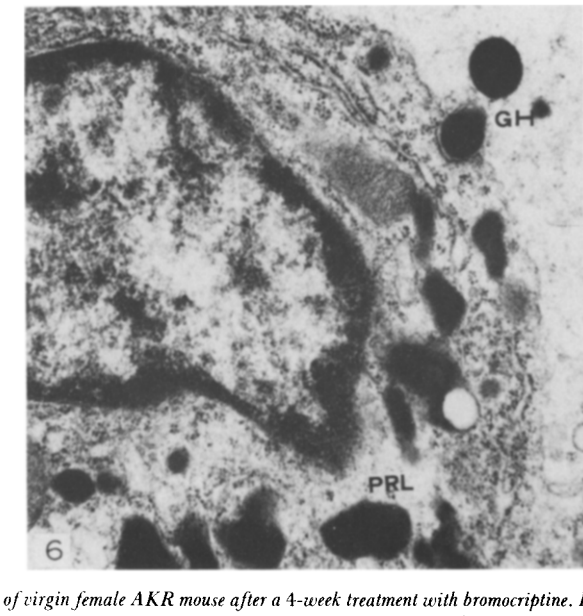

Fig. 6. Adenohypophysis of virgin female AKR mouse after a 4-week treatment with bromocriptine. Part of a 'new' cell type with mixed characteristics of somatotroph and lactotroph cells. ×24,000.

[13] who support the idea that the release rather than synthesis is affected. There is, however, so far, uncertainty as to whether the main action of bromocriptine takes place at the level of inhibition of prolactin release and/or inhibition of its synthesis.

Furthermore, some regressive changes were also observed. Atypical cells, with mixed characteristics of somatotrophs and lactotrophs, could be observed 4 weeks after the start of the treatment. These cells are not typical either for prolactin or for growth hormone but rather seemed to be somewhere in between to a cell of a lower differentiation. This 'new' type of cell was also described by Rossi [14] who reported, moreover, that typical lactotroph cells, commonly found in controls, were no longer found after bromocriptine treatment. This observation does not agree with our findings. It is probable that the 'new' cell type is a result of a dedifferentiating action of bromocriptine on lactotrophs to a cell of lower differentiation which may differentiate either to a somatotroph or to a lactotroph. Antakly *et al.* [13] report that lactotroph cells in culture treated with bromocriptine showed marked signs of regression.

There was no significant difference between the two strains of mice concerning the frequency of appearance of the 'new' cell type which was rather rare. It appears, therefore, that the regressive action of bromocriptine on lactotrophs is independent of the concentration of prolactin in the adenohypophysis.

Although we do not have absolute numbers of chromophobes, we observed that, by comparison with the untreated mice, they increased in the bromocriptine treated mice. Chromophobes presented no visible secretory granules and were once thought to be undifferentiated or resting cells without secretory activity [15].

In view of these data we may suppose that bromocriptine induces a dedifferentiation of lactotrophs with a subsequent increase of chromophobes cells and the appearance of the 'new' cell which we believe lies somewhere between the chromophobe cell and the somatotroph and lactotroph cell. Apparently, long term treatment of mice with bromocriptine seems to modify to a certain degree the pituitary morphology and function.

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