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MORPHOMETRY OF THE PITUITARY GLAND OF GROWTH HORMONE-TRANSGENIC MICE

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ABSTRACT

The pituitary glands of 30 transgenic mice (TM) expressing the bovine growth hormone (bGH) gene under the transcriptional control of the rat phosphoenol-pyruvate-carboxy-kinase promoter (PEPCK) as well as of 30 controls were investigated with stereological methods. The volume of the total pituitary, the adenohypophysis, and the neurohypophysis were estimated in paraffin sections using Cavalieri's principle. The numerical density of the growth hormone- (GH), prolactin- (PRL) and somatomammotropic (SOMA) cells as well as the size of the GH-cells were investigated on immunohistochemically stained sections. The volume fraction of all cell types was estimated using the point counting method. Mean volume, numerical density, and total number of the immunostained cells were measured by model-based stereological techniques following the method of Weibel and Gomez (1962). In comparing TM with age- and sex-matched controls, significant changes were detected: Both the weight and the volume of the pituitary glands were increased in female TM, whereas they were decreased in male TM. No differences were noted in the volume of the intermediate lobe or of the neurohypophysis. The volume fraction of GH- and SOMA-cells was reduced, both in male and in female TM, whereas the volume fraction of the PRL-cells was reduced in male TM and increased in female TM. The decrease of GH-immunopositive cells in TM was due both to a reduction in the number and in the size of somatotropic cells in GH-TM. These morphological data support the negative feedback mechanism caused by the protracted oversecretion of GH in various organs.

Key words: acidophilic cells, growth hormone, morphometry, pituitary gland, transgenic mice.

INTRODUCTION

Growth hormone-transgenic mice ectopically express foreign GH-genes and are especially suited for the investigation of the consequences of protracted exposure to excessive GH concentrations. TM show continuously high circulatory levels of heterologous GH and increased serum insulin like growth factor I (IGF I) concentrations. Stimulation of body and organ growth is their most evident phenotypic characteristic feature (Brem et al. 1989). Early pathological changes, noted in the kidney and in the liver, result in a drastic shortening of life span (Wolf et al. 1993). In addition, many endocrine disorders like reduced fertility or an increased adrenal activity can be observed. In this context, studies of different lines of GH-TM show functional and morphological changes in the pituitary glands (Stefaneanu et al. 1990).
The aim of the present study was to investigate by means of morphometry the histological changes of the pituitary gland of TM, with special regard to the acidophilic cells.

MATERIAL AND METHODS

TM were breeded and kept as described by Wolf et al. (1993). Fifteen male and fifteen female TM aged from 2 weeks to 10 months and corresponding nontransgenic controls were euthanized under ether-anesthesia. The pituitary glands were removed, weighed, fixed in 6% buffered formalin for 5 days, routinely embbeded in paraffin, and serial 3 µm sections were prepared. Sections separated by a distance of 30 µm were stained with the trichrom-method of Masson. Sections separated by a distance of 90 µm were used for the immunohistochemical detection of the acidophilic cells. The indirect immunoperoxidase-method for the detection of GH combined with the Avidin-Biotin-Complex-AP-method for the detection of PRL were considered to stain GH and PRL simultaneously.
The morphometric analyses were performed with the semi-automated image analysis system Videoplan (Kontron, Eching, FRG). The volumes of the embedded, i.e. shrunken pituitary gland and of its pars distalis, pars intermedia, and of the neurohypophysis were estimated following Cavalieri's principle (cf. Gundersen and Jensen 1987) by planimetry on trichrome stained sections. The calculated coefficient of error was less than 5%. The shrinkage was calculated as the volume of the embedded pituitary divided by the volume of the unfixed organ assuming a specific weight of 1g/cm^3. All parameters were corrected by this shrinkage factor.
The volume fraction (V_v) of the immunopositive cells was estimated by the point counting method (Oberholzer 1983) at a magnification of 1:1430 using an integrated test grid with 150 test points. The mean volume of the GH-cells ($\bar{V}_{GH}$) was estimated following the equation given by Hirose et al. (1982):

$$\bar{V}_{GH} = \beta/\kappa \times A^{1.5}$$

The value of the shape coefficient for elipsoids (ß) was dependent on the minimal and maximal cell diameter and was read from Fig. 2.30 given by Weibel (1979). The value of the size distribution coefficient (ϰ) was read from Fig. 2.31 given by Weibel (1979) considering the mean coefficient of variation (CV) for cell diameters. The mean profile area of GH-cells ($\bar{A}$) was measured by planimetry.
The numerical density/volume of the GH-immunopositive cells ($N_{V(GH)}$) was calculated as $V_{V(GH)}$ divided by $\bar{V}_{GH}$ and the total number of these cells (N_{tot}) was determined by multiplication of $N_{V(GH)}$ with the volume of the pars distalis. The selection of all test areas was perfomed with a three-dimensional systematic random sampling. For validation, $N_{V(GH)}$ was calculated according to the formula of Weibel and Gomez (1962) after determination of the numerical density/profile area (N_A) of the GH-cells using an unbiased counting frame of N_A-estimation.
Statistical analyses were performed applying the non parametric Mann-Whitney-U-test.

RESULTS

The differences between TM and controls are exemplarily depicted in Figs. 1 and 2 illustrating the results obtained from 4 months old animals (n = 3/group and sex). In animals aged two weeks, no differences in the body weights were observed, whereas in all other groups, the body weights of TM were higher than in controls ($p < 0.05$) (Fig.1).The volume of the anterior lobe of the pituitary gland was markedly increased in female TM, whereas the anterior lobes of male TM were smaller ($p < 0.05$) (Fig. 1). However, the pituitary gland weight/body weight ratio was markedly decreased in both sexes of TM. No differences were obtained in either the volume of the intermediate lobe or the neurohypophysis.

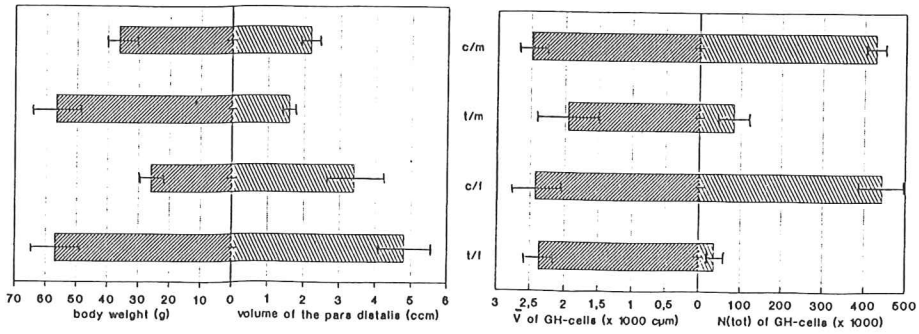

Fig. 1. Histograms showing body weight, volume of the pars distalis, size of the GH-cells ($\bar{V}$), and total number of the GH-cells in the pars distalis (N(tot)) of male controls (c/m), male TM (t/m), female controls (c/f), and female TM (t/f).

V_v of the somatotropic cells (Fig. 2) was significantly decreased ($p < 0.05$) in TM, which was already seen in the animal group aged 2 months. Also, V of the GH-immunopositive cells was markedly reduced in TM (Fig. 1). This reduction in cell size was primarily due to a reduction in the cytoplasm, whereas nuclear size was not changed. In addition, a reduction in N_v as well as in N_{tot} of the GH-immunopositive cells ($p < 0.01$) was detected in TM (Fig. 1). V_v of the PRL-cells was slightly increased in male TM as compared to male controls (Fig. 2). In female TM, however, an increase ($p < 0.05$) of the mammotropes was observed. Somatomammotropic cells, producing both GH and PRL, were detected in various amounts as high as 10% in the controls, but, in TM, they could not be demonstrated or their volume fraction was less than 1%.

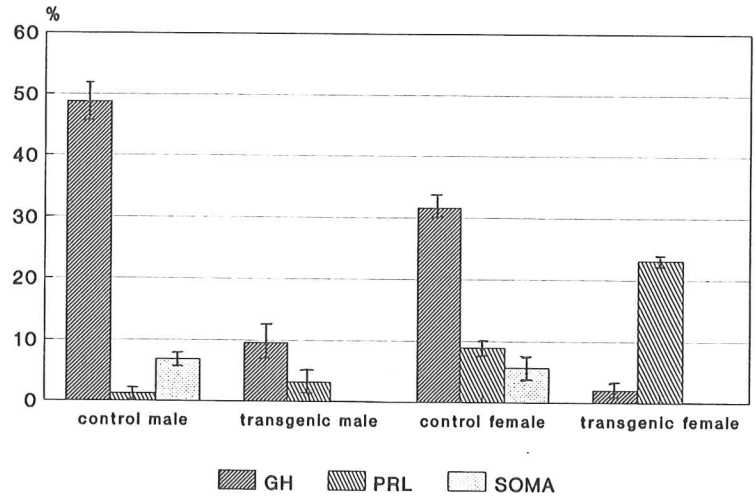

Fig. 2. Histogram showing the volume fractions (V_v) of GH-, PRL-, and SOMA-cells in the adenohypophysis of male controls, male TM, female controls, and female TM aged 4 months.

DISCUSSION

The purpose of the present study was to quantify the morphological alterations in the pituitary gland of GH-TM. Significant changes in the volume of the anterior lobe of TM were detected using Cavalieri's method. In order to elucidate these changes, the number and size of the acidophilic cells were specifically investigated.

As a result of the immunohistochemical staining and of the intrinsic characteristics of the pituitary gland, no design-based stereological methods could be applied. First, specific antibodies do not penetrate a thick paraffin section; therefore, the application of the optical disector (Gundersen et al. 1988, West and Gundersen 1990) was impossible. Second, the GH-immunopositive cells were very densely packed in the pituitary anterior lobe of controls, and, thus, an exact identification of the same cell in serial sections was

not possible. As a result, the physical disector could not be used as an alternative. Thus, model-based stereological methods had to be applied.

The results of the present study show that the volume changes of the pituitary anterior lobe of TM were primarily due to a reduction of the volume fraction of the GH-immunostained cells as a consequence of a reduction of both the number and the size of the somatotropic cells. These alterations may be explained by a drastic reduction of endogeneous GH as a consequence of a negative feedback caused by elevated foreign GH-levels and by elevated IGF 1 plasmic concentrations. A positive immunostaining was observed exclusively in the acidophilic cells of the pituitary anterior lobe. Thus, paracrine and autocrine effects of foreign GH gene expression can be excluded.

In addition to the alterations of the somatotropes, V_v of the mammotropes was also changed. The increase in PRL-immunopositive cells in female TM leading to an increased volume of the pars distalis is in accordance with results of Bartke et al. (1990), who detected elevated PRL blood concentrations. It is believed that a negative feedback of the heterologous GH may diminish the tuberoinfundibular turnover of dopamin, which is the most potent inhibitor of prolactin (Bansal 1981, Steger et al. 1991). However, this effect was only observed in female TM, but not in male TM, suggesting that sexual steroids may trigger the sensibility of the mammotropic cells to inhibiting signals. Obviously, these sex-specific differences in the mammotropic cell population account for the differences in the size of the pituitary anterior lobe between male and female TM.

The consequences of permanently elevated GH levels to somatomammotropic cells have not been investigated up to now. The marked reduction in somatomammotropes seems to be a further sign of negative feedback to GH-synthesis in TM.

Our results obtained from the pituitary glands of TM show that the combination of stereology with immunohistochemistry is well-suited to demonstrate the histologic substrate of functional metabolic changes. Further investigations on the brain and pituitary gland in TM will surely provide new insights into neuroendocrine regulation of the pituitary gland.

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