ACTA STEREOL 1994; 13/1: 21-26
PROC 6ECS PRAGUE, 1993
ORIGINAL SCIENTIFIC PAPER

SEASONAL VARIATION IN THE INFLUENCE OF PINEALECTOMY ON RAT FOLLICULAR AND PARAFOLLICULAR CELLS

Draga Štiblar-Martinčič

Institute of Histology and Embryology,
Faculty of Medicine, Korytkova 2, 61105 Ljubljana
Slovenia

ABSTRACT

The aim of this investigation, using systematical stereological analysis, was to test the hypothesis that pinealectomy increases thyroid gland activity and that this effect may vary with the season.

Two identical experiments were carried out, the first in summer and the second in winter. Each experiment was performed on 9 adult male rats of the Wistar strain, 3 of them being pinealectomized (P), 3 sham pinealectomized (SP) and 3 left intact as controls (C). After surgery all 3 groups were exposed to the natural light-dark rhythm for one month.

The summer experiment revealed significant differences between group P and C in the numerical volume density of follicular cells ($P \leq 0.01$) and in the average nuclear diameter of parafollicular cells ($P \leq 0.02$), in addition to the already published results.

In the winter experiment a significant increase in the numerical volume density of parafollicular cells ($P \leq 0.05$) was seen in group P as compared to group C, indicating a stimulating effect of pinealectomy on these cells, whereas significant increases in the epithelium volume density ($P \leq 0.05$), the thyroid gland activation index ($P \leq 0.02$) and the average volume of parafollicular cells ($P \leq 0.05$) observed in group SP as compared to group C could indicate a stimulating effect of trauma on the thyroid gland.

Regarding the seasonal variation in the effect of pinealectomy on the thyroid gland it is assumed that in winter the influence of trauma predominates whereas in summer pinealectomy has a stimulating effect.

Key words: pinealectomy, season, thyroid gland, rat.

INTRODUCTION

Some literature data indicate an increased activity of the thyroid gland when the inhibitory effect of the pineal gland has been eliminated by pinealectomy (De Fronzo and Roth 1972, Ishibashi et al. 1966, Šćepović 1963, 1965, and Miline et al. 1968).

However, observations concerning the influence of pinealectomy on the thyroid parafollicular cells, are inconsistent. McMillan et al.(1985), on the basis of pinealectomy experiments carried out in summer and winter, concluded that the pineal had little influence on the number and size of rat parafollicular cells. In an experiment terminated in summer, these authors observed a statistically insignificant decrease in parafollicular cells six weeks post surgery, no effect being seen at 12 weeks. On the other hand, a slight increase in the number of parafollicular cells was seen in January, 12 weeks post

surgery. No statistically significant differences were found between the groups, although the author used a combination of highly specific immunohistological technique and a morphometric method specifically designed for measuring the number and size of these cells.

Seasonal differences in basal hormone levels in the serum of adult male rats were studied by Wong et al. (1983). Their results indicate that male laboratory rats exhibit a circannual and semiannual fluctuation in serum levels of TSH, prolactin, androgens, triiodothyronine and LH.

On the basis of literature data and our previous investigations (Štiblar-Martinčič et al. 1992) we assumed that the influence of pinealectomy on the thyroid gland varies with the season, and we attempted to verify this effect.

MATERIAL AND METHODS

Two identical experiments were performed, the first in summer and the second in winter. In each experiment nine adult male rats of the Wistar strain, six months old, were divided into three groups with three animals in each. The animals of the first group were pinealectomized *(P)* (Šćepović, 1965), those of the second group were sham pinealectomized *(SP)* and those of the third group remained intact as controls *(C)*. After surgery all three groups were exposed to the natural light-dark rhythm for one month. Food and living conditions for all three groups were the same.

For light-microscopical analysis the thyroid gland together with the trachea were removed from the sacrificed animals and fixed in Bouin's solution. The thyroid glands were cut into 6 μm thick step serial sections, each step being 180 μm, stained with hematoxylin-eosin and PAS-light green, and impregnated with $AgNO_3$ after Fernandez-Pasquale (1976), in order to demonstrate the parafollicular cells.

Stereological analysis (Kališnik, 1985) was performed with a Wild sampling microscope, using Weibel's M-42 and M-100 test systems. The epithelium (V_{Ve}) and colloid (V_{Vc}) volume densities and the thyroid gland activation index *(IA)*, expressed as the quotient of the epithelium and colloid volume densities, were estimated at an objective magnification of x40, using the M-42 test system. In the parallel serial sections the volume density (V_V) of follicular and parafollicular cells , their cytoplasm (V_{Vcy}), nuclei (V_{Vn}), and numerical volume density (N_V) were estimated at an objective magnification of x40, using the M-100 test system. From the relative stereological variables the average and total values were calculated.

The results were statistically evaluated with the analysis of variance. Significant differences were determined with the Student "t" test.

RESULTS

There were no qualitative differences between the experimental groups. The following quantitative differences were revealed by the stereological analysis:

Summer

The summer experiment has been reported in a previous paper (Štiblar-Martinčič et al., 1992), where the results concerning the epithelium volume density, thyroid gland activation index, nuclear-cytoplasmic ratio and numerical volume density of follicular cells, as well as the volume density, nuclear-cytoplasmic ratio, average volume density and average nuclear volume density of parafollicular cells are discussed. In addition, a 60 % increase in the numerical volume density *(Fig.1)* of follicular cells and a 5 %

increase in the average nuclear diameter *(Fig.1)* of parafollicular cells were observed in group P as compared to group C. All these findings support the hypothesis that pinealectomy has a stimulating effect on the thyroid gland.

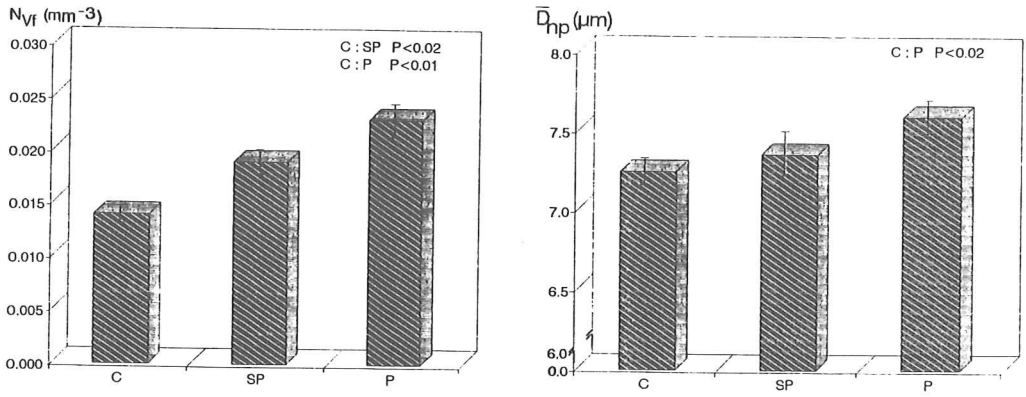

Fig.1. Numerical volume density of follicular cells (N_{Vf}) and average nuclear diameter of parafollicular cells ($\bar{D}_{np}$) for control (C), sham pinealectomized (SP) and pinealectomized (P) rats in summer ($\bar{x} \pm 1SE$).

Winter

The numerical volume density of parafollicular cells increased by 41 % in group P, as compared to group C *(Fig.2)*. This has been interpreted as the stimulating effect of pinealectomy on the thyroid gland.

Compared to group C, group SP showed a 37 % increase in the epithelium volume density *(Fig.2)*, a 42 % increase in the thyroid gland activation index *(Fig.3)*, and a 21 % increase in the average volume of parafollicular cells *(Fig.3)*; these results indicate a stimulating effect of trauma on the thyroid gland.

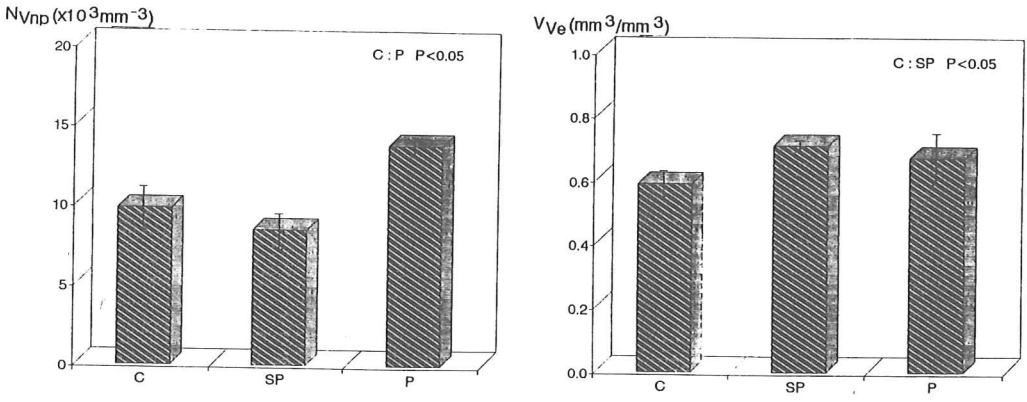

Fig.2. Numerical volume density of parafollicular cells (N_{Vnp}) and epithelium volume density (V_{Ve}) for control (C), sham pinealectomized (SP) and pinealectomized (P) rats in winter ($\bar{x} \pm 1SE$).

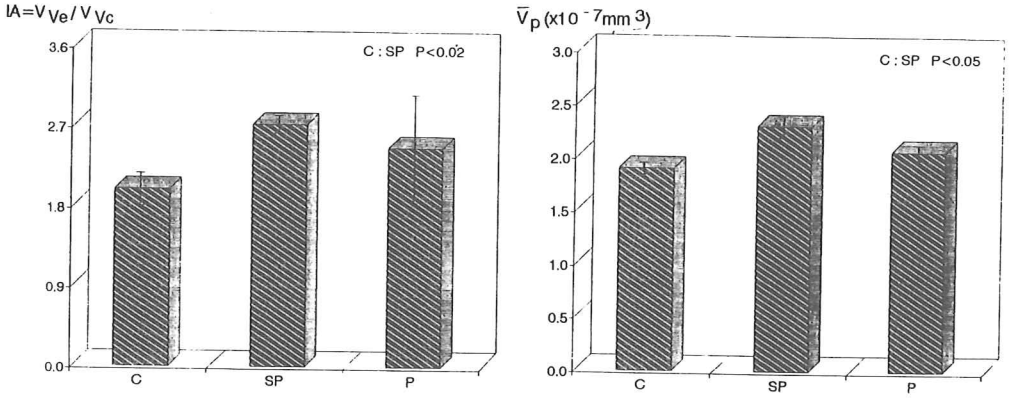

Fig.3. Activation index, expressed as the quotient of epithelium and colloid volume density ($IA = V_{Ve}/V_{Vc}$) and average volume of parafollicular cells ($\bar{V}_p$) for control (C), sham pinealectomized (SP) and pinealectomized (P) rats in winter ($\bar{x} \pm 1SE$).

DISCUSSION

The results presented indicate an increased activity of thyroid follicular and parafollicular cells following pinealectomy in the summer experiment (Štiblar-Martinčič et al., 1992).

The results of the experiment carried out in winter reveal a different response of the thyroid gland to pinealectomy. The numerical volume density of parafollicular cells was the only variable supporting our hypothesis of pinealectomy having a stimulating effect on the thyroid gland. The other stereological variables, including the thyroid gland activation index, epithelium volume density and average volume of parafollicular cells, showed a greater activity of the thyroid gland in the group of sham pinealectomized animals. We assume that the trauma or posttraumatic stress enhanced the activity of the gland.

Activation of the thyroid gland after disturbance stress was investigated by Döhler et al. (1977). Adult rats were decapitated without anaesthesia after disturbance stress, and serum concentrations of TSH, T3 and T4 were measured by radioimmunoassay. TSH and T3 levels were significantly elevated, whereas T4 showed only minor fluctuation during the experimental period. Johnston (1965), studying men who required inguinal herniorrhaphy, observed increased thyroid gland activity, evidenced by elevated T4 and T3 levels after the operation.

According to Petkó (1978) the number of parafollicular cells in the rat thyroid increases in autumn and decreases in spring. Unfortunately, these data are not quite reliable because the author did not describe the morphometric method used.

The results of the present stereological analysis confirm the hypothesis that pinealectomy has a stimulating effect on the thyroid gland. This effect seems to be modified by season, the influence of trauma being predominant in winter and that of pinealectomy in summer.

REFERENCES

De Fronzo RA, Roth WD. Evidence for the existence of a pineal-adrenal and a pineal-thyroid axis. Acta Endocr 1972; 70: 31-42.

Döhler KD, Gärtner K, Muhlen A, Döhler U. Activation of anterior pituitary, thyroid and adrenal gland in rats after disturbance stress. Acta Endocrinol 1977; 86: 489-97.

Fernandez-Pasquale JS. A new method for easy demonstration of argyrophil cells. Stain Technol 1976; 51: 231-5.

Ishibashi T, Hahn DW, Srivastava L, Kumaresan P, Turner CW. Effect of pinealectomy and melatonin on feed consumption and thyroid hormone secretion rate. Proc Soc Exp Biol 1966; 122: 644-7.

Johnston IDA. The effect of surgical operation on thyroid function. Proc Royal Soc Med 1965; 12: 1017-20.

Kališnik M. Fundamental stereology (in Slovenian). Acta Stareol 4/Suppl1: 1-148.

McMillan PJ, Heidbuchel U, Vollrath K. Number and size of rat thyroid C cells: No effect of pinealectomy. Anat Rec 1985; 212: 167-71.

Miline R, Šćepović M, Krstić R. Influence de l'épiphysectomie sur les cellules parafolliculaires de la glande thyroide. CR Assoc Anat 1968; 139: 893-8.

Petkó M. Seasonal variations of rat thyroid C cell population. Acta Biol Acad Hung 1978; 29: 367-74.

Šćepović M. Contribution à l'étude histophysiologique de la glande thyroide chez les rats épiphysectomisés. Ann Endocr (Paris) 1963; 24: 371-6.

Šćepović M. Histophysiological characteristics of thyroid gland and diencephalon after pinealectomy (in Serbian). Thesis, Sarajevo 1965.

Štiblar-Martinčič D, Kališnik M. The influence of pinealectomy on the microscopical structure of rat thyroid gland. Acta Stereol 1992; 11/Suppl1: 729-34.

Wong CC, Döhler KD, Atkinson MJ, Geerlings H, Hesch RD, Muhlen A. Circannual variations in serum concentrations of pituitary, thyroid, parathyroid, gonadal and adrenal hormones in male laboratory rats. J Endocrinol 1983; 97: 179-85.