
C CELLS IN THE THYROID OF PINEALECTOMIZED RATS

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PURPOSE: To study quantitatively C cells in the thyroids of non-isogenic rats to determine the possible effects of pinealectomy on the number of these cells, and consequently on the synthesis and secretion of calcitonin.

METHODS: Twenty male rats of an outbred strain (200-300 g) were used in the present study. One group of 10 animals was pinealectomized 50 days prior to sacrifice. Thyroid tissue was stained for calcitonin (Dako Corporation) at a 1:1500 dilution. The number of C cells observed was expressed as number of cells/cm². Data were analyzed statistically by Mann-Whitney test.

RESULTS: The number of C cells in pinealectomized and normal animals ranged from 489 to 2084 per cm² and 227 to 1584 per cm², respectively, a difference that was statistically significant ($P < 0.05$).

CONCLUSIONS: These results showed consistent differences in the number of C cells after pinealectomy when compared to controls. We believe that pinealectomy increases the number of C cells in the rat thyroid.

DESCRIPTORS: Thyroid. C Cells. Pineal Gland. Rat. Calcitonin.

INTRODUCTION

There is some evidence that the pineal gland is involved in various endocrine functions, and its effect on the thyroid has been investigated in particular, by several authors who suggest that melatonin inhibits thyroid function by suppressing the production of pituitary TSH¹⁻³, while others state that melatonin has a stimulatory effect^{4,5}, or that its effects on the thyroid are negligible^{6,7}. Thus, this is a highly controversial topic.

Few studies are available concerning the effect of the pineal gland on the metabolism and number of C cells⁸. Although some studies have shown an increase in the number of these cells⁹⁻¹¹, of their cytoplasm/nu-

cleus ratio and of the number of secretory granules¹² after pinealectomy (PX), McMillan et al.¹³ detected no significant differences in any of these parameters following PX.

In view of these considerations, the objective of the present investigation was to study C cells quantitatively in non-isogenic rats to determine the possible effects of PX on the number of these cells and consequently on the synthesis and secretion of calcitonin.

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MATERIALS AND METHODS

Sample section

The study was conducted on 20 non-isogenic male Wistar rats (*Rattus norvegicus*) weighing 200 to 300 g. The animals were divided into 2 groups: control (normal rats receiving sham operations, N = 10) and pinealectomized (N = 10).

Pinealectomy

Pinealectomy was performed using the method of Haldar-Misra¹⁴. The animals were weighed, anesthetized with sodium pentobarbital and placed in a stereotaxic apparatus for head fixation to facilitate the surgical procedure. The

region selected for craniotomy was shaved and disinfected, the skin was retracted with a separator, and the skull was exposed. Using a circular serrate burr, craniotomy was performed at the level of the lambdoid suture. The bone fragment was removed and placed in an appropriate vessel containing physiological saline. The sagittal sinus was separated, the pineal gland was fully removed by pinching, and the bone fragment was returned to its place. The various anatomical planes were then sutured. Both groups (control and pinealectomized) underwent prophylactic antibiotic treatment and were returned to their cages for recovery.

Sham surgery was performed following the same procedure as described above, except that the pineal gland was not removed.

Thyroid collection

Under inhalation ether anesthesia, the chest of the animals was opened and the heart exposed. The left ventricle was perfused first with physiological saline (0.9% NaCl) until blanching of the organs was achieved (requiring about 300 mL) and then with 4% buffered formalin, pH 7.4, until the animal stiffened (requiring about 100 mL). The pineal region was investigated, and the gland was completely removed. After perfusion, the thyroid was collected together with the trachea and placed in a glass vessel containing buffered formalin, pH 7.4. The interval between pinealectomy and removal of the thyroid was 50 days.

Thyroid analysis

After dissection and fixation in 4% formalin, the thyroid-trachea specimen was cut crosswise at 1 to 2 mm intervals, and all fragments were dehydrated in an increasingly concentrated ethyl alcohol series (70%, 80%, 90%, and absolute alcohol), cleared with

xylene, embedded in paraffin, and cut on a microtome into 4 mm-thick sections. The sections were then dried in an oven at 37°C; some of the slides were stained with hematoxylin-eosin, and some by the immunohistochemical technique for later histological analysis. The whole thyroid was studied with semi-serial sections.

Immunohistochemical technique

Anticalcitonin antibody at a 1:1500 dilution was used for immunohistochemical analysis. Sections were deparaffinized, hydrated, and pretreated with 0.01 M sodium citrate buffer in a microwave oven for 5 cycles of 3 minutes each at maximum power (900 W). The volume was completed with sodium citrate buffer in each cycle to prevent section drying. The slides were left to rest for 20 minutes and were then immersed in phosphate-buffered saline (PBS), pH 7.4, for 10 minutes. The sections were then incubated with normal horse serum for 20 minutes and covered with the antibody at the same dilution for 22 hours at 4°C. The avidin-biotin system was

used, and the material was processed using 3-amino-9-ethyl-carbazole (AEC A-5754, Sigma) as the chromogen. The sections were counterstained with Mayer hematoxylin, and a fragment of normal thyroid from an adult rat was used as positive control. The negative control was a sample of the case to be evaluated that had not been treated with the primary antibody.

Cell counts

The slides containing the thyroid fragments were submitted to microscopic analysis for C cell counts using a light microscope. The cells were counted in a 1 cm² area defined with a grid considering color (red, due to the action of AEC), morphology, and topography. The mean number of cells/field was determined for each case by 2 observers.

Statistical analysis

The Mann-Whitney test was used to compare the results between normal and pinealectomized animals, with the level of significance set at 5%.

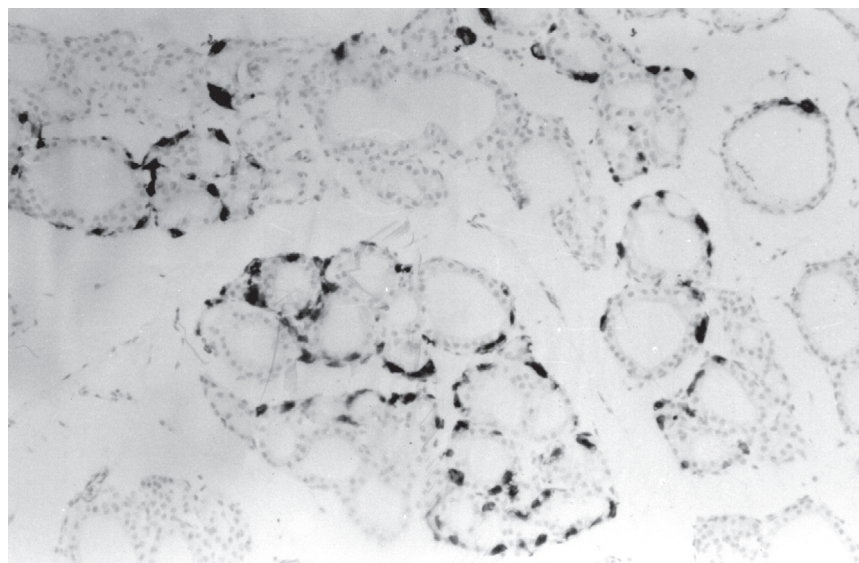


Figure 1 - Histological section of thyroid showing an increase in C cell number in pinealectomized rats (stained for calcitonin, 200x).

Ethics

This study conformed with the guiding principles of the Declaration of Helsinki.

RESULTS

The histological sections stained with hematoxylin-eosin did not show any alterations in thyroid morphology. When the immunohistochemical method was used, C cells, which were stained red-brownish, were concentrated in the lobes of the gland and were absent in the isthmus.

The number of C cells ranged from 227 to 1584 cells/cm² in the normal group and from 489 to 2084 cells/cm² in pinealectomized animals (Table 1). The data showed a statistically significant (*P* < 0.05) difference in C cell number in pinealectomized rats.

Table 1 - Number of C cells/cm² of thyroid in normal and pinealectomized rats.

Animal no.	Normal	Pinealectomized
R1	974	1755
R2	314	1261
R3	227	1374
R4	419	489
R5	1041	1145
R6	902	968
R7	1584	2084
R8	905	1387
R9	389	1295
R10	357	827

Table 2 - Analyses of variance (Mann-Whitney test) of the number of C cells/cm² between pinealectomized and normal animals.

Animals	Median C cells/cm ²	Minimum And Maximum	Sum Ranks	U Statistic	Mann-Whitney P value
Normal	661	227 1584	73	18	0,015
Pinealectomized	1278	489 2084	137		

DISCUSSION

The results obtained in the present study did demonstrate a statistically significant increase in C cells in the thyroids of the pinealectomized group. These data disagree with those reported by Brammer *et al.*⁷ and McMillan *et al.*¹³ in studies on rats in which they found no evidence of a significant interaction between the pineal gland and the hypothalamus-pituitary-thyroid axis. However, these results agree with those reported in other studies, which have suggested the influence of the pineal gland on the number of the C cells of the thyroid^{11,13}.

With respect to the arrangement of C cells, the observation that they were concentrated in the lobes of the gland and were practically absent in the isthmus has also been reported by McMillan *et al.*¹³, and our data confirm his report. The same author stated that there were no significant differences in C cell numbers between the right and left thyroid lobe.

Several studies have been conducted to define the relationship between the pineal and the thyroid. In 1966, Ishibashi *et al.* proposed that PX stimulates the release of thyroxin. In 1972, Relkin¹³ suggested that the pineal gland inhibits the secretion of thyroid hormones, and in 1976 he observed a reduction in plasma TSH levels after intraventricular injection of melatonin in the rat¹⁴. Malendowicz and Woznicki¹⁵ in 1986 supported this finding of an inhibitory effect of the pineal gland on thyroid function in a

study of the role of pinealectomy and melatonin in Wistar rats, as did Haldar and Pandey¹⁶ in 1988 in a study of pinealectomy in squirrels.

However, there is not a consensus among investigators regarding this inhibitory effect of the pineal gland on the thyroid. In 1970, Rowe *et al.*⁶ observed that PX did not affect plasma TSH levels. In 1979, Brammer *et al.*⁷ observed that the TRH content of the hypothalamus, the TSH content of the pituitary and plasma, and the serum levels of free T₃ and T₄ were not affected by PX. Additionally, Peschke *et al.*¹⁷ showed that PX may induce a state of prolonged hypothyroidism. Several studies^{18,19} have shown that physical stress such as anesthesia, surgery, and electric shock inhibits the thyroid-pituitary axis. Although transitory, the effect of PX on the response to TSH may be specific and may not be due to surgical stress. Barbot *et al.*²⁰ suggested that TSH regulates calcitonin synthesis in a manner similar to the regulation of thyroid hormones, and experimental studies^{21,22} have suggested that TSH hypersecretion may be responsible for C cell hyperplasia. Currently, we know that C cells, although inserted in the thyroid parenchyma, have an action that is independent of TSH and of the thyroid hormones.

The pineal gland could influence two important components of the calcium-regulating system in the body—that is, parathyroid and C cells—with an action that is at times stimulating and at times inhibitory^{9,10}. Animal data indicate that pineal melatonin is involved in the regulation of calcium and phosphorus metabolism by stimulating the activity of the parathyroid glands and inhibiting calcitonin release and prostaglandin synthesis²¹⁻²³. Hence, the pineal gland may function as a “fine tuner” of calcium homeostasis.

With respect to the number and activity of thyroid C cells, Krstic¹⁰ de-

tected hyperplasia and hypertrophy of these cells after PX. More recently, McMillan *et al.*¹³ compared the effects of PX and sham surgery in Wistar rats studied during different seasons of the year and observed no significant differences in the number or volume of C cells of rats undergoing PX or sham surgery, nor did they detect any important seasonal variation in these parameters.

Thus, we believe that the effect of the pineal gland on C cells may be discrete, with little influence on the number or size of these cells. However, the data obtained in the present study

regarding rat C cells will be valuable for future comparative and experimental studies.

On the other hand, the reduction in melatonin cannot be considered the only explanation for the effect of pinealectomy, since this hormone is also synthesized in the retina and in enterochromaffin cells^{26,27}. After PX, these tissues may gradually assume the role of the pineal gland.

Consequently, future studies should be performed to observe the effect of pinealectomy on C cell proliferation and function over time, contributing to the elucidation of the re-

lationship between C cells and the pineal gland. In summary, this study provides preliminary evidence for the potential efficacy of melatonin for controlling C cell proliferation, and possibly for co-treatment of medullary carcinoma. Therefore, it should be extended by other experiments related to both C cell proliferation and secretion.

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RESUMO

LIMA MA de e col. - Células C em tireóides de ratos pinealectomizados. **Rev. Hosp. Clín. Fac. Med. S. Paulo** 58(1): 21-26, 2003.

OBJETIVO: Estudar quantitativamente células C de tireóides em ratos

não isogênicos para determinar os possíveis efeitos da pinealectomia no número destas células e conseqüentemente na síntese e secreção de calcitonina.

MÉTODO: Vinte ratos machos com peso de 200 a 300g foram utilizados no presente estudo. Um grupo de

10 animais foi pinealectomizado 50 dias antes do sacrifício. Os cortes de tireóide foram corados com calcitonina (DAKO Corporation) a diluição de 1:1500. O número de células C observado é expresso como número de células/cm². Os dados foram analisados

pela análise de variância (ANOVA) e teste Mann-Whitney.

RESULTADOS: O número de células C em animais pinealectomizados e normais variou de 489 a 2084 células/cm² e 227 a 1584 células/cm², res-

pectivamente.

CONCLUSÕES: Estes resultados mostraram diferenças consistentes ($p < 0,05$) no número de células C após a pinealectomia quando comparada aos controles. Nós acreditamos que a

pinealectomia aumenta o número de células C nas tireóides dos ratos.

DESCRITORES: Tiróide. Células C. Glândula Pineal. Rato. Calcitonina.

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