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ORIGINAL ARTICLE

Hyperplasia of the colonic neuroendocrine cells after pinealectomy in rats.

The new evidence for the existence of connections between the distant parts of the Diffuse Neuroendocrine System.

Júlio César Fernandes da Silva, Marcelo Vinícius Oliveira Vespúcio, Renato Belini Dalio, Marco Aurelio Guimarães, André Péra Marques Pinto, Waynice Neiva de Paula Garcia, Sergio Zucoloto & Sérgio Britto Garcia

Department of Pathology, Ribeirão Preto School of Medicine, University of São Paulo, Brazil

Correspondence to: Dr. Sérgio Britto Garcia
Departamento de Patologia
Faculdade de Medicina de Ribeirão Preto
Universidade de São Paulo
Ribeirão Preto – Estado de São Paulo – BRASIL
EMAIL: sbgarcia@fmrp.usp.br

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Abstract

OBJECTIVES: The diffuse neuroendocrine system (DNES) participates in the systemic homeostasis and may work as a unified system with integrated functions but anatomically disperse. However, the mechanisms that are involved in the integration of the distant parts of the DNES are poorly known. This study is aimed on the effects of a pinealectomy on the population of the neuroendocrine cells (NECs) in the colon of rats.

METHODS: A group of seven newborn rats and one of adult rats were submitted to a pinealectomy. The animals were sacrificed after 15 days and 90 days, respectively. The control groups of adult and newborn rats were subjected to a "sham surgery" and then sacrificed after similar periods of time. Paraffin-included sections of the colon samples were stained by the Churukian-Schenk method in order to selectively stain the NECs. The NEC index was estimated by the expression: iNEC = NECs population per crypt/Total cell population of the crypt. The results were expressed as median and absolute range, and the statistical significance was demonstrated by the Mann-Whitney (U) test.

RESULTS: The morphometric analysis showed a significant increase in the number of colonic NECs of the adult rats subjected to a pinealectomy when compared to the controls. No statistical difference was observed between the iNECs of the newborn groups of rats.

CONCLUSION: This finding suggests the existence of regulatory mechanisms that integrate distant parts of the DNES, by adapting the population of the endocrine cells after the ablation of an important component of the system. Further studies on the mechanisms involved in the integration of the DNES may be facilitated by the simple experimental model that we propose.

Abbreviations

NEC – Neuroendocrine Cells
DNS – Diffuse Neuroendocrine System

Introduction

The neuroendocrine system of the vertebrates can be defined as a set of cells organized in single organs and diffuse elements, sharing the co-production of amine hormone/transmitters, peptide hormone/transmitters and specific markers of neural determination [18]. It is now recognized that the neuroendocrine characteristics can be observed in various cell types that are not of a common embryological origin with either neurons or endocrine cells [6]. Various theories, such as the APUD (amine precursor uptake decarboxylation system) concept, the diffuse neuroendocrine system (DNES) or the paraneuron concept have been put forth to classify the neuroendocrine cells as a cohesive group. This leads to the proposal that the nervous system and the endocrine system could be integrated and work as a unified system with integrated functions but anatomically disperse – the concept of the diffuse neuroendocrine system [13,14]. With cells present in almost all the organs of the body, this system may participate in the homeostasis regulation, via endocrine and paracrine [14]. However, the mechanisms that are involved in the integration of the endocrine cells in different organs are poorly known.

The peptide melatonin takes part in diverse areas of the human metabolism, such as the biological rhythms and the homeostatic actions [9]. Melatonin is also thought to be an essential substance in the DNES [9,12] and has been identified mainly in the pineal gland. However, it has been found in a wide variety of cells throughout the organism, including the enterochromaffin cells of the intestine mucosal lining which are also called the intestine neuroendocrine cells (NECs) [2,15,16]. Interestingly, it had been observed that a pinealectomy does not change the amount of melatonin in the gastrointestinal tract during the day [1] and that the gut contributes to maintain the melatonin circulating levels in the higher vertebrates [8]. However, it is not known whether the NECs possess some type of functional connection with the pineal gland.

We had previously shown that the gut myenteric denervation leads to a hyperplasia of the NECs [17]. This suggests that the population of NECs depends on the levels of the neurotransmitters and/or neuropeptides in the gastrointestinal wall. It is known that the autonomic nervous system contributes to the DNES [18] and that the pineal secretion is also known to affect the autonomic nervous system function and secretions [2,8]. Thus, our aim in this study was to verify the consequences of a pinealectomy on the colon NECs population.

Materials and methods

Male Wistar rats, supplied by the Ribeirão Preto School of Medicine, were housed upto five per cage in a temperature controlled room at 24 ± 1 and maintained on a 12:12-h light–dark cycle. The rats were provided with the same diet, a commercial chow purchased from the Ribeirão Preto School of Medicine, and tap water on an *ad libitum* basis. The animals were maintained in agreement with the guidelines of the Committee on Care and Uses of Laboratory Animals of the National Research Council of the N.I.H. (USA) and under the Brazilian government regulations.

Two groups of seven adult rats (with an approximated 150g of body weight) and two-groups of seven newborn rats were used for the experiment. The newborn rats were anaesthetized by hypothermia [18], subjected to a pinealectomy on the first day life and then they were sacrificed after 15 days (NP group).

The adult rats were anaesthetized by an intra peritoneal injection of 2,2,2 tribromoethanol (25mg/100g bw), subjected to a pinealectomy and then *sacrificed after 90 days*.

The control groups of newborn and adult rats (groups NC and AC, respectively) were subjected to a "sham surgery" and then sacrificed after the same time as the rats that were submitted to the pinealectomy.

The pinealectomy was performed as proposed by Wayforth [19] and the only difference between a pinealectomy and a sham surgery is that the pineal is not removed in the sham surgery.

All the adult animals were euthanized 90 days after the surgery by an inhalation of carbon dioxide saturated atmosphere and samples were collected from the descendent colon. The specimens were fixed in a 10% Bouin's solution and paraffin sections of 4 μm thickness were stained by the Churukian-Schenk method in order to selectively stain the gut NECs [12]. The sections were 40 μm far from each other in order to avoid analyzing the same crypt twice. The total cell population and the ECs population of 100 crypts were estimated by a cumulative mean [17].

The Endocrine cell index was calculated by the expression:

$$\text{iNEC} = \frac{\text{ECs population per crypt}}{\text{Total cell population of the crypt}}$$

Results were expressed as median and absolute range**,** and the statistical significance was demonstrated by the Mann-Whitney (U) test. A p value of < 0.05 was considered significant.

Results

All the animals remained in good health during the experimental period. At the time of the end of the experiment there was no body weight difference between the two groups of adult rats or the two groups of newborn rats, respectively. The histopathological

analysis showed that the colon morphology was normal in all the animals.

The morphometric analysis of the selectively stained NECs in the colonic crypts showed a remarkable increase in the number of colonic NECs of the adult rats subjected to the pinealectomy when compared to the adult rats submitted to the sham surgery, in both absolute and relative terms. Furthermore, there was no difference in the numbers of neuroendocrine cells between the two groups of newborn rats. (*Table 1*)

Table 1: The effect of a pinealectomy on the endocrine cell population index (iNEC) per 100 colonic crypts in adult rats, 90 days after the surgery and in new born rats, 15 days after the surgery. (**AP**= adult rats submitted to a pinealectomy, **AS**= adult rats submitted to a simulated surgery, **NP**= newborn rats submitted to a pinealectomy, **NS**= newborn rats submitted to a simulated surgery. Results are expressed as median and range (*p* significant ≤ *0,05*).

Group	iNEC	Range
AP	98	62
AS	58	40
NP	37	43
NS	38	20

iNEC: AP>AS (p=0.019) and NP=NS (p= 0,28)

Discussion

The observation described here, that a pinealectomy causes hyperplasia of the colonic NECs in adult rats suggests that there may be some sort of functional integration among the components of the DNES, which seems to be anatomically diffused in different organs, but could be able to act in a functional synergic way. Interestingly, this hypothesis may be in accordance to the concept of a recently proposed new acronym "Triune Information Network", which is proposed as an informational supersystem of the internal secretions acting in Mammals via progressively higher levels of the control of the DNES, which include the diffuse autocrine/paracrine/endocrine secretions, the autonomic nervous system and the hypothalamic-pituitary-target organ axis [18].

We also observed that the pinealectomy did not cause any changes the NECs population of newborn rats. This may be explained by the previous observation that the production of melatonin by the pineal gland is controlled by the darkness and, in rats, this production of melatonin is started seventeen day after birth because it depends on the maturity of the visual system of the animals [10]. All together, the results suggest that in our experimental model melatonin could have triggered the NEC hyperplasia in adult animals. From our findings we can only hypothesize on the mechanisms involved in the hyperplasia of the NECs after the pinealectomy.

Recently Day et al proposed to redefine the previous "neuroendocrine" concepts in including the notion that the activation of specific genetic switches can lead to the expression of a partial or full neuroendocrine phenotype in a variety of cell types, including the immune cells [6]. Melatonin has been considered as a central element of the Diffuse Neuroendocrine System, after the discovery of its endocrine and paracrine functions [7]. Functionally, melatonin-producing cells are certain to be part of the diffuse neuroendocrine system as a universal system of response, control and organism protection [10]. It is known that in adult rats the pinealectomy increases general cellular proliferation of the intestinal mucosae [3]. It has been proposed that hormonal mechanisms should be involved in this increase since the denervation of the pineal gland by ablation of the superior cervical sympathetic ganglia could not avoid this effect [4]. Similarly, from our findings it may be hypothesized that a decreased blood level of melatonin after the pinealectomy could trigger the hyperplasia of the NECs. Nevertheless, further studies are necessary to establish the mechanisms involved in the hyperplasia of the colonic NECs after a pinealectomy.

Taking into account the large number of melatonin-producing cells in many organs, the wide spectrum of the biological activities of melatonin and especially its main property as a universal regulator of the biological rhythms, Kvetnoy has proposed that it should be possible to consider the extrapineal melatonin as a key paracrine signal molecule for the local coordination of the intercellular relationships. Interestingly, it has been observed that the total number of gut NECs shows an increasing trend in the advancing age in rats [11]. Whether such increase can be related to the well established atrophy of the pineal that occurs during the aging process is an important subject to be investigated.

The Churukian-Schenk technique is an argirophilic technique that is able to identify the argirophilic cells that correspond to the neuroendocrine cells [5]. These cells may be classified in sub populations that are selectively able to produce serotonin, enteroglucagon, pancreatic peptid and somatostatin [7]. In our study we were not able to distinguish between these subpopulations of cells and further studies are warrant to refine the observations on the responses of the melatonin producer population of cells after a pinealectomy.

Further studies are needed to explain the mechanisms involved in the hyperplasia of the NECs and to refine the understanding on the mechanisms that may functionally integrate the parts of the DNES. Such studies may be facilitated by the simple experimental model that we propose.

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