A study of the effects of pinealectomy on intestinal cell proliferation in infant newborn rats[1]

Estudo dos efeitos da pinealectomia na proliferação celular intestinal de ratos recém-nascido

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ABSTRACT

Purpose: Study the proliferation rate of jejunum and large intestine crypt epithelial cells, in rats pinealectomized immediately after borning. **Methods**: Twenty-four male Wistar rats were distributed into two groups: Acute group (n=12) and Chronic group (n=12). Six animals of each group were operated for removal of the pineal gland (pinealectomy-PnX), and other six were controls (sham pinealectomy-C). Animals from acute and chronic group were sacrificed 15 and 90 days after the surgery, respectively. **Results**: In acute group, pinealectomy of new-born rats has not caused significant alteration in cell proliferation (PnX=58,77±1,77 and C=60,88±1,10 in the descending colon/ PnX=31,56±0,45 and C=31,73±0,47 in the proximal jejunum) and in crypt cell population (PnX=24,92±4,82 and C=23,60±2,48 in the descending colon/ PnX=39,92±3,49 and C=44,32±5,56 in the proximal jejunum). However, in chronic group there was an uprising crypt cell production per crypt in the proximal jejunum (PnX=57,54±2,19 and C=47,19±7,3)and in the descending colon (PnX=37,78±2,22 and C=17,92±2,28). **Conclusion**: As the increase of intestinal crypts epithelial cells in chronic group is a carcinogenesis predetermining factor, the understanding of the interaction between pineal gland and this event has great importance.

Key words: Pineal Gland. Intestine, Small. Instestine, Large. Infant, Newborn. Rats.

RESUMO

Objetivo: Estudar a taxa de proliferação celular no jejuno e nas células epiteliais das criptas do intestino grosso em ratos pinealectomizados imediatamente após o nascimento. **Métodos**: 24 ratos machos Wistar foram divididos em dois grupos. Grupo agudo (n=12) e Grupo Crônico (n=12). Seis animais de cada grupo foram operados para remover-se a glândula pineal (Pinealectomia-PnX), e outros seis animais foram controle (*sham* pinealectomia-C). Os animais de ambos os grupos foram sacrificados 15 e 90 dias após a cirurgia, respectivamente. **Resultados**: No grupo agudo, a pinealectomia dos ratos não causou alterações significativas na proliferação celular (PnX=58,77±1,77 e C=60,88±1,10 no cólon descendente / PnX=31,56±0,45 e C=31,73±0,47 no jejuno proximal) e na população celular de criptas (PnX=24,92±4,82 e C=23,60±2,48 no cólon descendente / PnX=39,92±3,49 e C=44,32±5,56 no jejuno proximal). Contudo, no grupo crônico houve aumento na proliferação celular das criptas no jejuno proximal (PnX=57,54±2,19 e C=47,19±7,3), e no cólon descendente (PnX=37,78±2,22 e C=17,92±2,28). **Conclusão**: Como o aumento epitelial celular das criptas intestinais no grupo crônico pode ser avaliado como fator predeterminante da carcinogênese, faz-se necessário o conhecimento da interação entre esta glândula e este evento.

Descritores: Glândula Pineal. Intestino Delgado. Intestino Grosso. Recém-Nascido. Ratos.

Introduction

The pineal gland has an important role on the control of the circadian rhythm and it is thought that it influences other general physiological processes such as hormonal controls[1]. There is growing evidence that the pineal gland also plays an important role on the control of cell proliferation. Callaghan et al [2], have observed that pinealectomy in adult rats causes a significant rise in the mitotic rate of the epithelial cells within the crypts of the small bowel, in a period from two to six months after the surgery. In the colonic crypts this hiperproliferative effect use to be smaller than in the small bowel. Mechanism that explains this effect is poorly known. Our aim was to study the proliferation rate of jejunum and large intestine crypt epithelial cells, in rats pinealectomized immediately after borning.

Methods

Twenty-four male Wistar rats were distributed into two groups: Acute group (n=12) and Chronic group (n=12). The animals were maintained at approximately 22ºC and in 12:12-h light-dark cycle, and supplied with tap water *ad libitum* and comertial food. One day after borning the animals were assigned randomly into two groups and anaesthetized with Nembutal. The acute group animals (n=12) were sacrificed 15 days after the surgery. Six animals of this group were operated for the removal of the pineal gland (pinealectomy), and other six were controls (Sham pinealectomy). The pinealectomy was performed via a scalp incision, a disc of bone was removed from the cranium over the lambdoid suture to expose the superior sagittal sinus, which was ligated and divided. The pineal gland was removed in a manner similar to described[3]. Bleeding was controlled by gelfoam (The Upkohn Co. Kalamazoo, Michigan, U.S.A) and the scalp was closed. The removed pineal gland was fixed in 10% phosphate-buffered formalin. Paraffin sections of 5 m thickness were stained with 1% toluidine blue and examined microscopically to confirm pinealectomy in all groups. The other rats were operated for a simulated surgery and did not have their gland extracted. The same experimental design was adopted to chronic group, with two subgroups: 1) Pinealectomized rats (CP) and 2) Control rats (CC). The same procedure as for pinealectomy was followed, including ligation of the superior sagittal sinus, except that the pineal gland was not removed. On the right date, the rats were killed with ether anesthesia, according to the metaphases blocking method with vincristine sulfated described later. The samples of colon and jejunum were embedded in paraffin. Five ìm thick sections oriented to show the long axis of the colon crypt were stained Hematoxylin-Eosin. Cell counts were made with a 40x objective and 10x ocular. The crypt cell population (CCP) was calculated as twice the hemicrypt population added to mitoses population. Thirty different crypts of each animal were utilized to the cell counting. The mitotic index (MI) of crypts was calculated by the expression:

$$MI = \frac{Nm \times Tf}{Nt}$$

Where Nm = number of mitoses (metaphases), Nt = total crypt cell population and Tf = Tannock's factor[4]. The mean MI value was plotted against time after vincristine injection. The resulting metaphases arrest line was fitted by least squares linear regression and the slope of the line multiplied by 60 minutes represented the cell production per crypt. This was expressed as cells /1000crypt cells/ hour. The results are reported as means ± SEM (Standard Error Mean), with the level of significance set at 5%.

Results

We observed that there was no alteration in proliferation of mucosa epithelium cell of pinealectomized animals from the acute group (15 days) when compared to the control groups, in colon (Figure 1) as much as jejunum (Figure 2).

Chronic group (90 days) There was clear increase of cell proliferation rate in the pinealectomized animals, compared to control groups, in colon (Figure 3) as much as in jejunum (Figure 4).

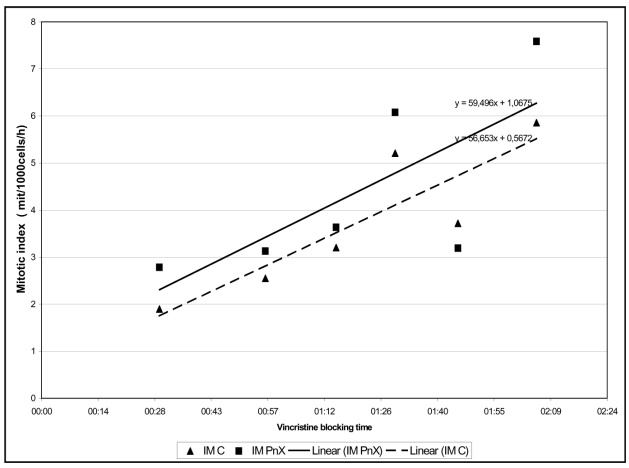

FIGURE 1 - Acute group mitotic index of colonic mucosa epithelium to each rat plotted against his respective vincristine blocking times.

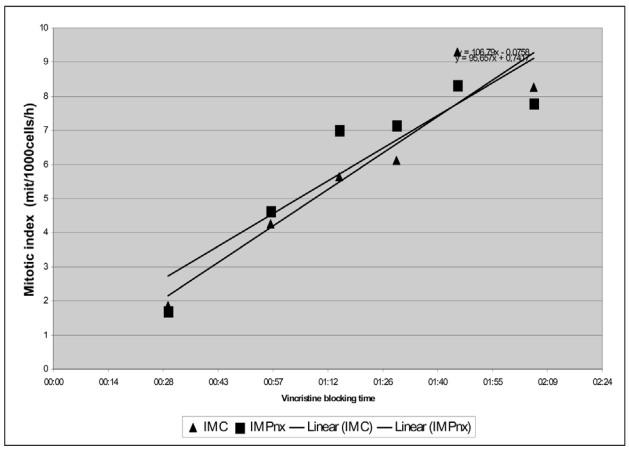

FIGURE 2 - Acute group mitotic index of jejunal mucosa epithelium from each rat, plotted against their respective vincristine blocking times.

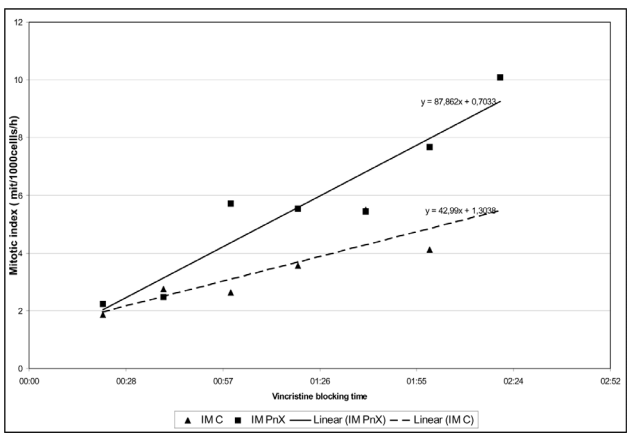

FIGURE 3 - Chronic group mitotic index of colonic mucosa epithelium from each rat, plotted against their respective vincristine blocking times.

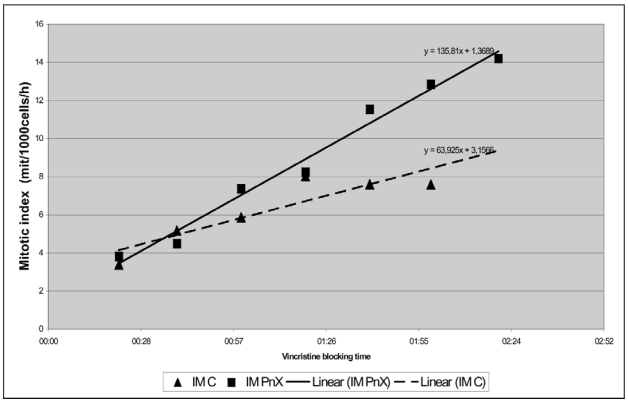

FIGURE 4 - Chronic group mitotic index of jejunal mucosa epithelium from each rat, plotted against their respective vincristine blocking times.

In Table 1, crypt cell population (CCP) and crypt cell production per crypt (CCPC) of colon in acute (15days) and chronic (90days) group are related. The results from proximal jejunum proliferation study are expressed in Table 2.

Analyzing table 1 and table 2, we can note that CCP has not expressive variation between C and PnX groups. In chronic group, CCP also has not expressive changed, however CCPC has showed a significant statistical variation. As observed in tables, results are worth to both experiments (descending colon and proximal jejunum).

TABLE 1 - Effect of pinealectomy in new-born rats over crypt cells population (CCP) and crypt cell production per crypt (CCPC) of descending colon in both acute and chronic groups. The results showed were obtained using arithmetic mean ±SEM (Standard Error Mean).

Group	Crypt Cell Population			Crypt Cell Population per Crypt*		
	PnX	Control	*p*	PnX	Control	*p*
Acute	58.77 +/- 1.77	60.88 +/- 1.10	>0.05	24.92 +/- 4.82	23.60 +/- 2.48	0.24
Chronic	107.61 +/- 5.58	96.50 +/- 1.54	>0.05	37.78 +/-2.22	17.92 +/- 2.28	0.027

*metaphase cells /1000 cells per crypt per hour
p >0.05 =significative

TABLE 2 - Effect of pinealectomy in new-born rats over crypt cells population (CCP) and crypt cell production per crypt (CCPC) of proximal jejunum in both acute and chronic groups. The results showed were obtained using arithmetic mean ±SEM (Standard Error Mean).

Group	Crypt Cell Population			Crypt Cell Population per Crypt*		
	PnX	Control	*p*	PnX	Control	*p*
Acute	31.56 +/- 0.45	31.73 +/- 0.47	>0.05	39.92 +/- 3.49	44.32 +/- 5.56	0.35
Chronic	70.21 +/- 1.94	70.05 +/- 2.22	>0.05	57.54+/- 2.19	47.19 +/- 7.3	0.005

*metaphase cells /1000 cells per crypt per hour
p >0.05 =significative

Discussion

Pinealectomy of new-born rats has not caused expressive alteration in cell proliferation and in crypt cell population, in acute group. However, in the chronic group we found an uprising crypt cell production per crypt in proximal jejunum, the same occurred in descending colon. Fifteen days aged rats do not have its optical tract pathways fully mature and mielinized yet. Once optical tract pathways integrity and maturity are fundamental to physiological function of pineal gland[5], we can infer that in this age the control made by this gland is also immature and less efficient, what justifies the results obtained. Another conceivable explanation for different degree of effect of pinealectomy in the small bowel and the colon may be that the effect is due to long standing changes in the local levels of a neurotransmitter that is more plentiful in the small bowel than in the colon may be that the effect is due to long standing changes in the local levels of a neurotransmitter that is more plentiful in the small bowel than in the colon, e.g, somatostatin[6]. However, there is no available experimental evidence that pinealectomy can effect the levels of these neurotransmitters. It is known that pinealectomy reduces the level of circulating melatonin by 80%[7] and that melatonin is not only produced by the pineal but it is also found in the gastrointestinal tract[8]. In fact, Menendez-Pelaez[9] have suggested a dual system in which a basal melatonin synthesis occurs in peripheral tissues, e. g, small and large bowel, while the circadian rhythm of melatonin is provided by the pineal gland. Thus, after pinealectomy, it is conceivable that the reduction in the level of melatonin in the colon might be less than in the

jejunum if they each individually produced different amounts of melatonin, e.g, if the colon produced relatively more melatonin than the small bowel. Melatonin production is relatively higher in the more proximal parts of the gastrointestinal tract[10]. We hypothetized three theoretical models for the action of pineal gland in the intestinal epithelium proliferation. They are presented in Figure 5 and described below.

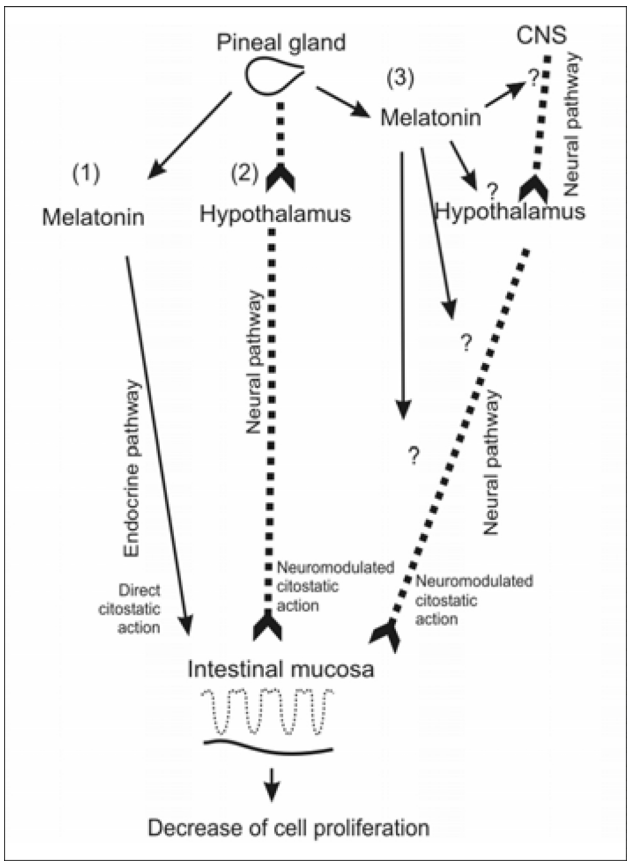

FIGURE 5 - Proposed mechanism to explain the effect of pineal gland in colonic and jejunal crypt cell proliferation control in rats. (1) Endocrinal action model, (2) Neural action model, (3) Combinated model

Model 1: Endocrine pathway – Plasmatic melatonin has endocrinal effect over colonic and jejunal mucosa cells, making direct citostatic action in cell proliferation. Pinealectomy leads to decrease in the plasmatic melatonin levels[11]. So, once in down levels, the citostatic effect of melatonin will not occur anymore, taking to an increasing cell proliferation. This model is believed by many authors. Melatonin has an inhibitory effect in jejunal and colonic epithelial cell proliferation of adult rats[12]. However, some researches deny this model. Intestinal neuroendocrinal cells also synthetize melatonin in a larger scale than pineal does[13]. This melatonin has a local action over mucosa cells. Melatonin levels in the gastrointestinal tract do not depend on pineal gland secretion, based on the fact that gastrointestinal melatonin levels do not change with pinealectomy[11].

Model 2: Neural pathway – Pineal gland could have a modulatory effect over jejunal and colonic crypt cells through nervous connections not already described. The pineal gland citostatic effect probably follows this sequence: 1) Direct connections between hypothalamus and pineal gland, 2) Connections between hypothalamus and intestinal autonomic innervation, 3) Intestinal autonomic innervation influence on the activity of enteric plexus over crypt cell proliferation[14]. Supporting this hypothesis, is known that hypothalamus injuries are related to significant intestinal mucosa cell proliferation increase, what leads to conclude that these changes are not worth to functional alterations in the endocrinal system[15]. So, an important portion of this physiological pathway is eliminated, acting the same way as a hypothalamous injury, increasing intestinal cell proliferation.

Model 3: Combinated action – Putting together pros and cons from the two models set before, a unifying hypothesis for a role of both neural and endocrine systems to explain the pineal gland control over intestinal cell proliferation can be formulated. Central nervous system (CNS) has, through hypothalamus and autonomic nervous system, an important role in control of jejunal and colonic mucosa cell proliferation. An example for that is the decrease of distal colon tumor incidence when denervated by benzalkonium chloride (BAC)[16]. The melatonin released in plasma by pineal gland has an inhibitory modulator neuroendocrinal role in some place of nervous connection linking CNS and intestinal mucosa. Thus, pinealectomy promotes decreasing melatonin plasmatic levels, priving the nervous pathway from its inhibitory control, leading to an intestinal cell proliferation increase. Some additional considerations can be done about the neural pathway and the combinated action. Some organs of the human body have close relations with other. Neural processes of the hipothalamic nuclei can terminate on fenestrated vessels of the portal venous system that carries factors to the anterior lobe of pituitary hormones. There isn't descripition of any similar device that provides priority to any region of the nervous system by melatonin. On the other hand, there are connections between the pineal gland and hipothalamus[17,18]. In addition, either hypothalamus injuries[15] or pinealectomy make to increase intestinal mucosa cell proliferation. These factors make the neural pathway specially attractive. As the increase of intestinal crypts epithelial cells is a carcinogenesis predetermining factor[19,20], the understanding of the interaction between pineal gland and this event has great importance. To turn this relationship clear, we intend to study these aspects following our research line on a brief future.

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Conflito de interesse: nenhum
Fonte de financiamento: nenhuma

Received: September 13, 2005
Review: October 19, 2005
Accepted: November 10, 2005

How to cite this article:
Dalio MB, Haikel Jr LF, Dalio RB, Pinto, APM, Silva JCF, Vespúcio MVO, Guimarães MA, Garcia SB. A study of the effects of pinealectomy on intestinal cell proleferation in infant newborn rats. Acta Cir Bras. [serial on the Internet] 2006 Jan-Feb;21(1). Available from URL: http://www.scielo.br/acb