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ORIGINAL ARTICLE

Changes in pineal sympathetic innervation are not significant in the hyperproliferative effects of pinealectomy on the intestinal crypts

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Abstract

OBJECTIVES: To observe the effect of changes in the pineal sympathetic innervation on the crypt cell proliferation rate in the rat small intestine, and compare these with the effect of pinealectomy to determine the role of the sympathetic innervation in the effect of pinealectomy.

METHODS: The effect of bilateral ablation of the superior cervical ganglion, and that of pinealectomy on the crypt cell proliferation rate in the rat small intestine was determined, using a stathmokinetic technique.

RESULTS: Pinealectomy was associated with a considerably increased crypt cell proliferation rate, whereas superior cervical ganglionectomy was associated with a slightly decreased rate.

CONCLUSIONS: It appears likely that changes in pineal melatonin production cannot be correlated directly with the effects of pinealectomy on the crypts, although melatonin production was not measured in this case. The role of loss of the non-adrenergic innervation of the pineal in the effect of pinealectomy needs to be examined. There is also other experimental evidence that melatonin-free extracts of the pineal, containing as yet unidentified substances, can influence mitotic activity in some tissues, so the possible role of these substances in the effects of pinealectomy should also be considered. Furthermore, the superior cervical ganglion itself has an extra-pineal role. Changes in pineal sympathetic innervation are not significant in the hyperproliferative effects of pinealectomy on the intestinal crypts.

Abbreviations:

SCG x	Superior cervical ganglionectomy
h	Hours
p	Probability
df	Degrees of freedom
t	Value of "t" distribution

Introduction

It has been shown [1] that the principal innervation of the pineal gland by sympathetic nerve fibres arising in the superior cervical ganglion is essential for its function.

It has also been shown [2] that this sympathetic innervation influences melatonin metabolism in the mammalian pineal gland, and that melatonin can both stimulate and suppress rat crypt cell proliferation [3, 4].

It has also been found that excision of the pineal gland (pinealectomy) is associated with a marked increase in the rat small bowel crypt cell proliferation rate [5, 6] and that the autonomic innervation of the gut is directly involved in this effect [7]. If the superior cervical ganglion plays a major role in the control of crypt cell proliferation, via the sympathetic nervous system, bilateral excision of this ganglion (with decreased pineal melatonin secretion) would be expected to have a similar qualitative and quantitative effect on crypt cell proliferation as pinealectomy.

Accordingly, I have compared the effect of bilateral excision of the superior cervical ganglion and the effect of pinealectomy on the crypt cell mitotic rate in the rat small bowel, using a stathmokinetic technique.

Materials and Methods

21 male albino Sprague-Dawley rats weighing 325–475 gms were supplied by the Waite Institute, University of Adelaide, Adelaide, Australia. They were randomly selected into 4 groups and maintained at approximately 22° in light from 0.08 to 0.22 h in the Animal House. They were fed M and V mouse cubes (Charlick and Sons, Adelaide, Australia) and water ad libitum. All were anaesthetised with nembutal (Abbott Labs., Sydney, Australia).

In the first group (5 rats), via a midline incision, the sternomastoid muscles were divided, and the carotid sheath with the surrounding nerves was exposed on both sides. The superior cervical ganglion was removed bilaterally and the wound was closed (ganglionectomy).

In the second group (5 rats) the ganglion was exposed but not removed, and the wound was closed (sham-ganglionectomy).

In the third group (6 rats) the pineal gland was removed as previously described [8] and the superficial tissues were closed (pinealectomy).

In the fourth group (5 rats) the pineal was exposed but not removed (sham-pinealectomy).

Histological examination of the removed tissue, stained with Nissl stain, confirmed ganglionic structure. The removed pineal glands were stained with 1% toluidine blue to confirm pinealectomy.

Postoperative pain relief was ensured by local infiltration of the skin and superficial subcutaneous tissues with 2% xylocaine (Astra, Sydney, Australia) and rats were housed in separate cages. In all cases, penicillin (Commonwealth Serum Laboratories, Melbourne, Australia) was administered postoperatively.

After 2 weeks all rats subjected to superior cervical ganglionectomy, or sham-ganglionectomy, were killed by an overdose of ether (Merck, Pty, Ltd, Melbourne, Australia).

Rats subjected to pinealectomy or sham-pinealectomy were killed in the same manner after 3 weeks. Specimens were taken from 5 cm distal to the duodeno-jejunal junction (proximal jejunum) and 5 cm proximal to the ileocaecal junction (distal ileum) and stained with haemotoxylin and eosin.

Using a stathmokinetic technique [9] involving colchicine (Sigma Chemical Company, St. Louis, Mo., USA) in a dose of 0.1 mg / 100 gm of body weight, counts of metaphase figures were made on each tissue specimen in the lowest 20 cells of each crypt (excluding Paneth cells). 40 crypts were counted for each specimen of gut, and the mitotic index calculated. The mitotic rate was determined for each tissue specimen from the relationship between the mitotic index and the duration of colchicine treatment, and expressed as mitoses / cell / hour.

The statistical significance of apparent differences between the mitotic rates was estimated by means of Student's two-tailed t-test. A P value < 0.05 was considered significant.

Results

In rats subjected to pinealectomy the mitotic rates in the proximal jejunum and distal ileum, respectively, exceeded the rates of those subjected to sham-pinealectomy (proximal jejunum, $p < 0.05$, $df = 7$, $t = 20.26$; distal ileum, $p < 0.05$, $df = 7$, $t = 27.15$, Table 1).

In rats subjected to pinealectomy the mitotic rates in the proximal jejunum and distal ileum, respectively, exceeded the rates of those subjected to superior cervical ganglionectomy (proximal jejunum, $p < 0.05$, $df = 7$, $t = 11.61$; distal ileum, $p < 0.05$, $df = 7$, $t = 21.44$, Table 1).

Table 1: Mitotic rates in the rat small intestine following superior cervical ganglionectomy or pinealectomy (mitoses / cell / hour) (means ± SEM).

Lesions	Ganglionectomy (n= 5)	Sham-ganglionectomy (n = 5)	Pinealectomy (n = 6)	Sham-Pinealectomy (n= 5)
Proximal Jejunum	0.0830 ± 0.0041	0.0929 ± 0.0023	0.1283 ± 0.0017	0.0756±0.0019
	|-------- p < 0.10 --------|		|-------- p < 0.05 --------|	
Distal Ileum	0.0710 ± 0.0023	0.0820 ± 0.0016	0.1289 ±0.0017	0.0773±0.0010
	|--------- p < 0.01 --------|		|-------- p < 0.05 --------|	

The mitotic rates in the distal ileum of rats subjected to sham-ganglionectomy exceeded those in the distal ileum of rats subjected to ganglionectomy ($P < 0.01$, $t = 4.07$, $df = 6$, Table 1). The mitotic rates in the proximal jejunum of rats subjected to sham-ganglionectomy exceeded those in the proximal jejunum of rats subjected to ganglionectomy, but not statistically significantly ($P < 0.10$, $t = 2.15$, $df = 6$, Table 1).

Discussion

The hyperproliferative effect of pinealectomy after 3 weeks is consistent with the effect after 2 weeks [10] but denervation of the pineal, by bilateral excision of the superior cervical ganglia, does not have the same hyperproliferative effects on the crypts. In fact, denervation of the pineal gland, with a decrease in its melatonin secretory function, resulted in a fall in crypt cell proliferation rate.

Previous investigations [11] suggest that it is unlikely that regeneration of the sympathetic innervation would have been present at 2 weeks. Thus, it appears unlikely that the superior cervical ganglion plays a major role in any possible neural mechanism of control of crypt cell proliferation which involves both the pineal and the sympathetic nervous system.

However, it does not rule out the participation of the remaining non-sympathetic innervation of the pineal in the neural control of crypt cell proliferation. Although melatonin metabolism in the mammalian pineal gland is under the influence of the sympathetic innervation, not all pineal cells are under sympathetic control [12].

It has been shown that various types of non-sympathetic nerve fibres of central origin supplying the pineal remain intact after superior cervical ganglionectomy [13]. These include peptidergic [14, 15, 16, 17], and histaminergic [18] fibres. The loss of this innervation after pinealectomy may be responsible to some extent for the difference in the effects on the crypts between these two procedures.

The crypt cell proliferation rate was decreased after ganglionectomy (statistically significant only in the ileum), and it is possible that this is due to the unopposed action of the non-sympathetic innervation of the pineal on the crypts. It is consistent with the findings that superior cervical ganglionectomized rats developed less carcinogen-induced mammary tumours [19].

In other ways the effects of pinealectomy do not always mimic those of superior cervical ganglionectomy (SCG,x) [20] so that SCGx should not be regarded as merely "pineal denervation". In fact, it has been suggested that the superior cervical ganglion has functional links with the medial basal hypothalamus and may act as a peripheral neuroendocrine centre [21]. It was also found [22] that SCGx affected differentially dopamine and indoleamine metabolism in the medial basal hypothalamus. Following SCGx, peripheral sympathetic nerve terminals in the median eminence were found to degenerate, and it has been suggested that peripheral sympathetic nerve terminals may modulate the acute stress responses of luteinizing hormone and corticosterone in rats [23]. Evidence has also been found [24] that the cervical autonomic nerves constitute a pathway through which the brain modulates calcium homeostasis, and that the pineal gland does not participate in short-term changes of parathyroid hormone or calcitonin release. Thus activity of the SCG can produce significant far-reaching effects besides its effect on the pineal gland

Furthermore, even if SCG x resulted in a fall in the level of melatonin secretion from the pineal gland this may not be sufficient to affect the proliferation rate in the mucosa, since the gastrointestinal tract is itself a rich source of melatonin [25]. However, melatonin produced by the pineal reaches the circulation immediately, whereas melatonin produced in the GIT reaches the peripheral circulation only occasionally [26] and functions mainly in the autocrine or paracrine capacity [27].

Thus, it is possible that pinealectomy may not produce its effect on the mucosa primarily by means of a fall in serum melatonin, and in support of this, the effect of pinealectomy on the crypts appears to be largely mediated via the gut autonomic nerve supply [7].

Although melatonin levels in the gastrointestinal tract are not reduced after pinealectomy, serum levels are [28]. Similarly, SCGx results in a fall in pineal melatonin production [29]. However, these 2 procedures have opposite effects on crypt cell production, and this would also suggest that changes in pineal melatonin production are not important in the production of changes in crypt cell proliferation.

On the other hand, although SCGx results in a decrease in pineal melatonin [29], it was found that the pineal gland of a ganglionectomized rat continues to secrete minimal amounts of melatonin [30]. Thus, it is possible that a critical level of melatonin is still produced after SCGx, sufficient to prevent serum melatonin levels falling to a level where the effects of melatonin in decreasing cell proliferation in the crypts would not be operative. Thus, pineal melatonin production may possibly be a factor in preventing hyperproliferation of the crypts.

Furthermore, since pineal melatonin secretion is under superior cervical ganglionic control, and SCGx removes only pineal melatonin secretion, another possibility can be considered to explain the differing effects of SCGx and pinealectomy on the crypts. It is possible that whereas SCGx removes pineal melatonin secretion, pinealectomy may be associated with the removal of additional pineal secretions, other than melatonin. In support of this possibility it has been found [31] that melatonin-free pineal extracts containing as yet unidentified pineal substances have potent tumour inhibiting activity.

Thus it appears that, although the superior cervical sympathetic ganglion does not play an important role in any possible adrenergic mechanism of control of crypt cell proliferation involving the pineal gland, the possible role of the non-adrenergic innervation of the pineal should be further investigated, as should the role of non-melatonin substances produced by the pineal gland It is also suggested that while it is possible that changes in pineal production of melatonin following pinealectomy are a factor in the hyperproliferative effects on the mucosa, it is more likely that the effect of pinealectomy on the crypts is mediated largely directly via the autonomic nervous supply to the gut, rather than via changes in pineal melatonin production. However, it is also possible that changes in local production of melatonin in the gut are involved in this effect.

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