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The pineal gland and cancer
I. Pinealectomy corrects congenital hormonal dysfunctions and prolongs life of cancer-prone C3H/He mice

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Abstract

Hormonal derangements almost invariably anticipate and signal the onset of tumors. Chronic, nocturnal melatonin administration delays aging in normal strains of mice. On the contrary it promotes and accelerates the onset of tumors in the cancer-prone strain of C3H/He mice. Grafting of a young pineal gland into aging mice prolongs their longevity and maintains juvenile circadian hormonal functions while pinealectomy (Px) does the opposite. We investigated if Px in C3H/He mice would modify their congenitally deranged pituitary function and affect their longevity. It was found that contrarily to Px in normal mice, Px in C3H/He mice remarkably maintains juvenile night levels of thyroid hormones and lipids, preserves a cell-mediated immune response and significantly prolongs their life. The pineal gland and its pathology may be the key for understanding, not only the causes of metabolic aging, but also the origin of those congenital or progressive aging-related hormonal alterations preceding onset of all tumors and thus allow preventive corrective interventions with pineal-derived agents.

Keywords: Pineal; Melatonin; Cancer; Pinealectomy; Lipids; Thyroid hormones

1. Introduction

In the course of previous studies we have observed that, contrarily to that found in other inbred strains of mice where chronic nocturnal administration of melatonin affects longevity and delays aging (Pierpaoli and Regelson, 1994), the cancer-prone strain of C3H/He mice, when given chronically melatonin in their nocturnal drinking water, develops a very high incidence of cancer of the reproductive tract and other kind of tumors such as reticulum cell sarcomas (Pierpaoli et al., 1991). It was thus clear that night administration of melatonin starting at 1 year of age, not only failed to prolong the life span of the mice, but even accelerated their death. The cancer-prone C3H strain of mice is known to carry congenital and age-progressing alterations of hormonal functions, in particular an increased output of prolactin and growth hormone from their pituitary gland (Sinha et al., 1975; Sinha et al., 1979). These mice develop a high number of spontaneous tumors, especially mammary carcinomas (Sinha et al., 1979). Our findings on the cancer-enhancing and/or accelerating effects of nocturnal melatonin administration in adult female C3H/He mice, and the demonstration that these mice show an abnormal shift of their night melatonin peak and production towards early morning (Goto et al., 1989), suggested to us an evaluation if the congenital alteration of their hypothalamic–hypophyseal, neuroendocrine regulation might also be under pineal control. In other words, we wanted to investigate whether pinealectomy (Px) could, contrarily to other strains of mice, improve survival and correct endocrine dysfunctions in these mice in the course of their aging and, consequently, prevent cancer and in general prolong their life. The results illustrated here indicate that Px in aging C3H/He mice produces (a) improvement and normalization of thyroid function, (b) a lowering of lipid levels in peripheral blood, and (c) maintenance of cell-mediated

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immunity, these positive changes resulting into a significant prolongation of their longevity.

2. Materials and methods

2.1. Animals

Young (4-month-old) inbred female BALB/cJ mice and inbred female C3H/He (10-month-old) mice bred and maintained under conventional conditions at our laboratory were used. Room temperature was 20–22°C, illumination was 07:00 h lights on, 19:00 h lights off. The animals were kept in groups of five per cage and checked twice daily. Mice were fed ad libitum with NAFAG (Gossau, Switzerland) maintenance fodder for mice, and tap water.

2.2. Pinealectomy (px)

Px was performed in young, 4-month-old inbred female BALB/cJ mice and in 10-month-old inbred female C3H/He mice by transcranial thermocoagulation under barbiturate anaesthesia (Vetanarcol). Absence of the pineal gland was confirmed in all mice by post-mortem macroscopic autoptic examination and in doubtful cases by light microscopy scrutiny with serial sections of the tissues surrounding the pineal area.

2.3. Pineal implantation into the thymus

An intact pineal gland from young donor (5-month-old) inbred female BALB/cJ mice was implanted into the thymus of aging inbred female BALB/cJ mice (18-month-old) pinealectomized at 4 months of age. Pineal implantation into the thymus was performed as previously described (Pierpaoli et al., 1991; Pierpaoli and Regelson, 1994).

2.4. Determination of thyroid hormones and lipids

For total triiodothyronine (T3), total thyroxine (T4) and lipid determinations, the mice were bled from the retroorbital venous plexus under acute ether anaesthesia at 1 a.m. under dim red light illumination. Sera from individual mice were kept separate and frozen at −30°c until the hormones were measured by radioimmunoassay (T4-Amerlex-M and T3-Bridge, Serono). Cholesterol, phospholipids and triglycerides were measured with an Hitachi 737 analyser.

2.5. Lifespan and cause of death

The dead mice were recorded twice daily and autoptic examination was performed within 1–12 h from death.

2.6. Assessment of delayed-type hypersensitivity (DTH) response

DTH response to oxazolone was assessed by application of 4 μl of 5% oxazolone (Aldrich Chemical Co., Milwaukee, WI) dissolved in acetone/oil (1:1, v/v) to the clipped skin of the chest and upper abdomen. Four days later, the mice were challenged by topical application of 25 μl 0.5% oxazolone on both sides of the right ear. DTH response was assessed by measuring the increase in ear thickness of oxazolone-sensitized mice 48 h after challenge with a modified micrometer dial gauge (Verdict Gauge Ltd., Dartford, Kent, UK).

3. Results

3.1. Lifespan and cause of death of Px and non-Px C3H/He mice

The findings illustrated in Table 1 show that Px of C3H/He female mice at 10 months of age, contrarily to chronic nocturnal administration of melatonin (Pierpaoli et al., 1991), significantly prolongs the longevity of the mice. The autoptic, post-mortem examination of both Px and non-Px C3H/He mice showed that, in the Px mice, the cause of death could not be attributed to the typical spontaneous tumors ensuing in this strain of mice, in particular mammary carcinomas in the female. The long-surviving Px C3H/He mice died of different causes including infections, renal insufficiency (nephrosclerosis), lung emphysema and other causes attributable to normal aging. On the contrary, a high percentage of the non-Px mice (60%) were affected by mammary carcinoma and other tumors, including ovary carcinomas and reticulum cell neoplasia.

3.2. Determination of lipids and thyroid hormones

T3 and T4 levels in blood follow a circadian cyclicity. The low *night* values normally increase in the course of aging thus producing a flattening of the juvenile day–night changes (Lakatua et al., 1984). When blood was taken at a constant time in the night and lipids and thyroid hormones were determined, it can be seen that at 1.5 months after Px in the 11.5-month-old C3H/He mice, the effect of Px on

Table 1
Pinealectomy prolongs the life span of C3H/He female mice[a]

Treatment	No. of mice	Survival (days)
Pinealectomy	15	880.1±51.0*
Controls	15	792.0±48.7

[a] Mean±S.D. * $P<0.01$ when compared to controls (Student's *t*-test). Female C3H/He mice were pinealectomized at 10 months of age by transcranial thermocoagulation under barbiturate anesthesia.

Table 2
Early effect of pinealectomy (PX) on night levels of T3, T4 and lipids in C3H/He female mice[a]

Parameters measured	Pinealectomy (n=9)	Controls (n=9)
Cholesterol (nmol/l)	2.65±0.31	2.62±0.51
Triglycerides (nmol/l)	2.62±0.53	2.63±0.56
Phospholipids (nmol/l)	3.13±0.24	2.92±0.79
T3 (ng/100 ml)	0.48±0.11	0.62±0.09*
T4 (μg/100 ml)	3.56±0.36	4.12±0.45*

[a] Mean±S.D. * $P<0.05$ when compared to pinealectomized mice (Student's t-test). Female C3H/He mice were pinealectomized at 10 months of age by transcranial thermocoagulation under barbiturate anesthesia; 1.5 months after pinealectomy, mice were bled from the retroorbital venous plexus under acute ether anaesthesia and dim red light at 1 a.m.

levels of lipids in the blood is not yet visible, while significant decrease of thyroid hormones can be observed (Table 2). When the same determinations were performed 8 months after Px in the 18-month-old mice, it was seen that Px produced a significant diminution of cholesterol and night levels of thyroid hormones, when compared to the non-Px C3H/He mice (Table 3).

In order to see if similar effects of Px could be observed in another hormonally normal strain of mice *different* from the pineal-deranged, cancer-prone C3H/He mice, an experiment was performed with BALB/c mice. As a confirmation of that observed previously also in the hormonally normal C57BL/6 mice (Pierpaoli et al., 1991), it can be seen that when BALB/c mice are Px at 4 months of age and examined at 21 months of age, profound changes occur in their thyroid function and lipid levels, expressed by elevated night levels of T3, T4 and lipids, in particular cholesterol (Table 4). These negative effects of Px on thyroid function are observed in BALB/c mice whether Px is performed at 4 or at 10 months of age (personal unpublished observations). When groups of the Px BALB/c mice are pineal-grafted at 18 months of age with a pineal from a young donor, pineal grafting positively corrects the deranged and abnormally high night levels of thyroid hormones. Also contrarily to the positive effects of pineal removal on lipid levels in the C3H/He strain (Table 3), restoration of pineal function by young-pineal grafting in the Px BALB/c mice corrects the high night levels of cholesterol produced by Px in BALB/c mice to the values of normal controls (Table 4).

Table 3
Late effect of pinealectomy (PX) on night levels of T3, T4 and lipids in C3H/He female mice[a]

Parameters measured	Pinealectomy (n=14)	Controls (n=15)
Cholesterol (nmol/l)	2.54±0.25	2.80±0.24*
Triglycerides (nmol/l)	1.74±0.29	1.98±0.57
T3 (ng/100 ml)	0.52±0.13	0.65±0.10*
T4 (μg/100 ml)	3.93±0.34	4.34±0.41*

[a] Mean±S.D. * $P<0.05$ when compared to pinealectomized mice (Student's t-test). Female C3H/He mice were pinealectomized at 10 months of age by transcranial thermocoagulation under barbiturate anesthesia. Eight months after pinealectomy, mice were bled from the retroorbital venus plexus under rapid acute ether anaesthesia and dim red light at 1 a.m.

Table 4
Effect of implantation of pineal glands from young donors into the thymus of pinealectomized senescent BALB/cJ recipients on night levels of T3, T4 and lipids[a]

Parameters measured	Controls (n=7)	Pinealectomized (n=7)	Pinealectomized +pineal graft (n=7)
Cholesterol (nmol/l)	2.20±0.45	2.74±0.30*	2.10±0.35
Triglycerides (nmol/l)	1.20±0.29	1.40±0.22	1.15±0.28
T3 (ng/100 ml)	0.71±0.13	0.92±0.08*	0.62±0.07
T4 (μg/100 ml)	2.56±0.86	3.48±0.42*	2.48±0.79

[a] Mean±S.D. * $P<0.05$ when compared to controls or pinealectomized+pineal graft (Student's t-test). Female BALB/cJ mice were pinealectomized at 4 months of age by transcranial thermocoagulation under barbiturate anesthesia. At the age of 18 months, groups of pinealectomized, senescent mice were grafted with an intact pineal from 5 month old syngeneic female BALB/cJ donors into the involuted thymus (see Sections 2 and 3). Three months after pineal grafting; age-matched controls, pinealectomized and pinealectomized-pineal grafted mice were bled at 1 a.m. under dim red light from the retroorbital venous plexus under acute ether anesthesia. Individual sera were kept separate at −30°C until determination of T3 and T4 by radioimmunoassay and determination of lipid levels.

3.3. Delayed-type hypersensitivity response (DHT)

When a typical parameter of a cell-mediated and thymus-dependent immune response was assayed, it was seen that Px produced an improvement of cell-mediated immunity of C3H/He mice (Table 5).

Table 5
Pinealectomy of C3H/He female mice maintains the delayed-type hypersensitivity (DTH) response[a]

Treatment	No. of mice	DTH response to oxazolone	
		Before challenge	After challenge
Pinealectomy	13	27.1±1.2**	34.0±1.7 (+25.4%)*
Controls	15	26.9±1.6	31.4±2.0 (+16.7%)

[a] Mean±S.D. * $P<0.05$ when compared to controls after challenge; ** P=NS when compared to controls before challenge (Student's t-test). Female C3H/He mice were pinealectomized at 10 months of age by transcranial thermocoagulation under barbiturate anesthesia. Mice were immunized with oxazolone 12 months after pinealectomy.

4. Discussion

We have shown that chronic melatonin administration in the nocturnal water significantly delays aging of BALB/c mice while it increases and accelerates onset and growth of carcinomas in the notoriously cancer-prone C3H strain of mice (Pierpaoli et al., 1991; Pierpaoli and Regelson, 1994). On the other hand, young-to-old pineal grafting remarkably prolongs the longevity in different strains of mice (Pierpaoli et al., 1991; Pierpaoli and Regelson, 1994). The findings reported above demonstrate that, contrarily to what observed in a normal inbred strain of mice (BALB/c) where Px produces a derangement of neuroendocrine functions and an acceleration of aging (Zwirska-Korczala et al., 1991; Mocchegiani et al., 1996), Px significantly and positively affects longevity (Table 1) and improves metabolic and hormonal conditions of the cancer-prone and genetically hormonally deranged strain of C3H/He mice, when those aging-related parameters are measured at 1.5 and 8 months after Px. (Tables 2 and 3). On the contrary, Px contributes to an acceleration of aging-related hormonal changes in the normal non-cancer prone strain of BALB/c mice, as shown by the high night levels of thyroid hormones and cholesterol which are typical of aging (Table 4). Those Px-induced changes can be totally corrected by young pineal grafting (Table 4). Therefore, Px in the hormonally deranged and cancer-prone strain of C3H/He mice produces a positive correction (decrease) of the night levels of thyroid hormones and cholesterol (Table 2 and 3), with highly significant prolongation of their lifespan (Table 1) and maintenance of cell-mediated immunity (Table 5), contrarily to what seen on cholesterol and thyroid hormone levels of BALB/c mice (Table 4) and of normal Px rats (Cunnane et al., 1979; Kniazewski et al., 1990).

These results are not surprising in view of the repeated observation that genetic endocrine imbalances, or chemically produced or spontaneous hormonal derangements almost inevitably precede or accompany the onset of a variety of tumors. In relation to mammary carcinomas, which are a frequent and spontaneous occurrence in the C3H strain of mice, it has been shown that, in fact, specific abrogation of growth hormone production with antibodies directed against growth hormone producing cells in the adenohypophysis can drastically reduce methylcholanthrene-induced mammary carcinoma in susceptible Sprague–Dawley female rats (Pierpaoli and Sorkin, 1972). Also evident is the role of endocrine disorders in the onset of systemic neoplasia in the SJL/J strain of mice (Pierpaoli et al., 1974). The host endocrine status mediates oncogenesis in leukemia virus-induced reticulum cell sarcomas (Pierpaoli and Meshorer, 1982) and hormonal treatments or a permanent modification of the host hormonal milieu via masculinization or gonadectomy drastically increase onset of leukemias and other types of tumors (Pierpaoli et al., 1977). It has also been shown that tumor incidence in C3H/HeN mice (mammary tumor virus positive) varies according to their thyroid state. Hyperthyroid C3H/HeN mice develop a high incidence of mammary tumors while in the hypothyroid groups the onset of mammary tumors is significantly lower (Vonderhaar and Greco, 1982). This data is consonant with the positive effects of Px on night thyroid hormones levels in the C3H/He strain used by us.

Pinealectomy disturbs the T3 diurnal rhythm and causes relevant changes of T4 levels during the 24 h (Kniazewski et al., 1990). In fact, in the experiments reported above, Px produced a maintenance of juvenile low night levels of T3 and T4 in C3H/He mice (Tables 2 and 3) while, on the contrary, Px produced an acceleration of the aging-related increase of the thyroid night levels in the BALB/c mice (Table 4), which is a consequence of the progressive age-dependent flattening of the normal circadian periodicity of thyroid function (Lakatua et al., 1984). Apparently, the maintenance of hormonal and in this case thyroid circadian rhythmicity is a fundamental aspect of cancer prevention. The demonstration that pineal grafting in the normal BALB/c strain corrects the deranged lipid and thyroid function produced by Px favours our hypothesis that the pineal gland controls and monitors most if not all circadian endocrine periodicity from birth until late age. A congenital or produced alteration of this basic cyclicity results either in early aging and/or cancer. In fact Px abolishes the rhythmic character of corticosterone secretion and disturbs the circadian rhythm of T3, T4 and testosterone (Zwirska-Korczala et al., 1991) while nocturnal, exogenous melatonin administration or young pineal grafting in aging mice and rats remarkably maintains peripheral (gonads) and central (hippocampus) integrity of the reproductive tract (Pierpaoli et al., 1997).

The night peak of melatonin has been shown to be shifted towards early morning in the C3H/He strain of mice (Goto et al., 1989). This genetic hormonal alteration may explain the normally high night levels of T3, T4 and lipids in C3H/He mice which are corrected by Px (Tables 2 and 3) and which seem to be typical of the C3H/He strain (Goto et al., 1989). The role of the pineal gland and circadian rhythms in controlling levels of thyroid hormones had already been observed in male hamsters (Vriend, 1984).

Our experiments were aimed at studying, not the onset of spontaneous tumors, but the role of the pineal gland on some fundamental hormonal and metabolic dysfunctions typical of aging in cancer-prone mice. The results show clearly that the pineal gland of C3H/He mice is probably responsible for the congenital derangement of hormonal functions in the cancer-prone strain of C3H/He mice. This strain shows high levels of prolactin which has been considered to be the main cause for the onset of mammary carcinomas (Pierpaoli and Sorkin, 1972). Without denying the role of a deranged hypophyseal growth hormone and prolactin secretion for the onset of cancer in the C3H/He

strain of mice, we suggest that a congenital or induced alteration of pineal function as reflected by profound alterations of the normal circadian cyclicity of basic glands such as the thyroid may be the key for interpreting high-risk cancer incidence in animals and humans.

In this respect melatonin may be an element in the control of a normal circadian cyclicity of hormones. However, we tend to believe that melatonin is not itself a key element for the pineal control of cancer (Regelson and Pierpaoli, 1987) but rather represents a *signal and marker* for aging and for congenital, induced (e.g. with chemical agents) or accelerated (e.g. with stress, irradiation etc.) pathological changes of the pineal gland. We ascribe to the the idea that other pineal-derived molecules, their deficiency or excess in the pineal gland, may be the answer (Pierpaoli et al., 2000).

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