Research report

Pineal metabolic reaction to retinal photostimulation in ganglionectomized rats

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Abstract

The aim of the present work was to test the pineal gland metabolic reactivity to nocturnal retinal short term photic stimulation in superior cervical ganglionectomized rats. The experimental support for this work is the appearance of a transitory post synaptic hyperactivity in the pineal gland, during the anterograde degenerating process of the conarii sympathetic nerve fibers after surgical removal of the cell body. In this situation the pineal gland is deafferented from the peripheral sympathetic nervous system keeping intact, however, the direct central connections to the deep pineal/lamina intercalaris region DP. The results show a blockade of the pineal noradrenergic stimulatory process due to the retinal photostimulation. The inactivation of N-acetyltransferase led to a true metabolic shift to the oxidative pathway resulting in a decrease of the amount of N-acetylserotonin and an increase of the amount of serotonin, 5-hydroxyindoleacetic acid and 5-hydroxytryptophan. This inhibitory process brought into action by retinal illumination is dependent on the direct central neural connections to the pineal gland, since rats that were lesioned in the DP, previously to ganglionectomy, did not show any alteration on the indolic content of the pineal gland when subjected to nocturnal retinal photostimulation.

Keywords: Pineal gland; Superior cervical ganglion; Ganglionectomy; Deep pineal; Lamina intercalaris; Intergeniculate leaflet

1. Introduction

The pineal gland, through the synthesis and releasing of melatonin, plays the role of interface between the cyclic environment and the rhythmic vertebrate body. The functional characteristic of the neuroendocrine system that controls the pineal gland makes the circadian production and secretion of melatonin tightly related to the dark phase of the diurnal environmental lighting cycle in order to signal to the internal milieu daily and seasonal timing cues [1,2,4,5].

In mammals, light stimulus, acting through the retina, can entrain the daily nocturnal peak of activity of the rate-limiting enzyme arylalkylamine N-acetyltransferase (NAT) responsible for the synthesis of N-acetylserotonin (NAS) and, consequently, melatonin [15]. In addition to this entraining role, brief pulses of light applied during the scotophase of the daily light/dark cycle are able to acutely inactivate pineal NAT, blocking the production and release of NAS and melatonin [12,16].

The neural pathway involved in the visual control of the metabolism of the mammalian pineal gland originates in the retina projecting through the retina-hypothalamic tract to the anterior perichiasmatic hypothalamus [13,19,36] mainly to the suprachiasmatic nucleus. The connection between this anterior retinal recipient hypothalamic area and the pineal gland is made through a neural system [24] that, at least, involves the paraventricular hypothalamic nucleus, the spinal intermediolateral cell column and the cervical sympathetic nervous system.

In addition to this peripheral sympathetic innervation, direct central projections to the pineal gland have been demonstrated [23]. These projections, originating from several sources (the hypothalamic paraventricular nucleus, the lateral hypothalamic zone, the tuberomammillary nucleus, mesencephalic raphe nuclei and the intergeniculate leaflet of the lateral geniculate thalamic complex), have as common terminal field the deep pineal/lamina intercalaris region (DP) of the epithalamus.

In previous work [6,7], we demonstrated that complete bilateral thalamic intergeniculate leaflet (IGL) lesion (either
electrolytic or ibotenic acid) produced a reduction in the daily peak of N-acetylserotonin in the pineal gland, and blocked the pineal NAT inhibition induced by nocturnal short term retinal photic stimulation. Moreover, it was demonstrated that the integrity of the DP terminal field of the direct central projection was necessary for the full expression of the nocturnal inhibitory phenomenon triggered by brief pulses of light.

The aim of the present work was to study, in superior cervical ganglionectomized rats, the reactivity of the pineal gland metabolism to acute short-term light pulses delivered at dark phase of the diurnal light/dark cycle.

The experimental support for this proposition is the appearance of a transitory post-synaptic hyperactivity in the pineal gland, in ganglionectomized rats, during the anterograde degenerating process of sympathetic nerves fibers after surgical removal of the cell body, situated in the superior cervical ganglion [3,25]. These data from the literature and preliminary experiments done in our laboratory, indicate that from 12 to 30 h after the ganglionectomy, the pineal gland metabolism is shifted to the acetylation region. Therefore, it is possible in this preparation to test the inhibitory effect of light mediated by direct neural connections to the pineal gland without the interference of the neural system that has the peripheral sympathetic system as the final effector.

The results show that nocturnal short-term pulses of light are able to block the pineal transitory noradrenergic stimulatory process in the sympathetic deafferented pineal gland without the interference of the neural system that has the direct central projection was necessary for the full expression of the inhibitory phenomenon triggered by brief pulses of light.

2. Materials and methods

2.1. Subjects

Adult male albino Wistar rats (200–220 g, n = 44) were kept, since weaning, under 12:12 h light/dark cycle (200 to 300 lux at cage level, white fluorescent bulbs: Kodak red 1A filter, 0.5 to 1.0 lux; lights on at 06.00 h) in a temperature-controlled (21 ± 2°C) room, with water and food available ad libitum.

2.2. Surgery and experimental procedures

In one type of experiment (three independent experimental blocks, n = 28, SCGx), 12 to 30 h before the experimental night, the animals were anesthetized with pentobarbital (35 mg/kg b.wt., i.p.) and the superior cervical ganglia were excised bilaterally under direct microscope vision. On the following night the animals were assigned to one of two experimental groups, paired according to the time of surgery. One group was subjected to 15 min of light (white fluorescent, 500 lux) stimulation 5 h after lights off and sacrificed immediately afterwards. The other group was, at the same time, manipulated as the photostimulated one but kept in the dark, being killed immediately after 15 min.

In another experimental design (two independent experiments, n = 16, DP-lesioned SCGx), 1 week before ganglionectomy the animals had an electrolytic lesion put into the deep pineal/lamina intercalaris region. They were anesthetized with sodium pentobarbital (35 mg/kg b.wt., i.p.), placed in a stereotaxic apparatus (David Kopf Instruments, California, USA) and small holes were drilled in the skull to allow penetration of the lesion electrode (epoxyite insulated stainless steel insect pin 00.03 mm of bare tip). The lesions were aimed at 4.8 mm posterior to bregma and 3.7 below the dura, at sagittal plane [27]. An anodal DC current of 2 mA was passed for 10 s (DC LMS Lesion Maker, Grass Instruments Co., Quincy, MA, USA), with the cathode attached to the skin of the back. Similarly to the first type of experiment, 12 to 30 h after ganglionectomy, the animals were divided into two groups and paired according to the time of the ganglionectomy. One group was kept in the dark, another group was subjected to 15 min light stimulation and immediately sacrificed.

The pineal glands were immediately removed after the completion of the experimental procedure, frozen in dry ice and kept in a −70°C freezer until assayed.

2.3. Chromatography

Tryptophan (TRY), 5-hydroxytryptophan (5-HTP), serotonin, 5-hydroxyindoleacetic acid (5-HIAA) and N-acetylserotonin were assayed by HPLC liquid chromatography with electrochemical detection, as described before [6]. In summary, each gland was sonicated and after centrifugation the clear supernatant (20 μl) was injected into the chromatographic system (Waters, Milford, MA, USA, composed of a 510 HPLC pump, a Resolve 5 μm spherical C18, 3.9 × 150 mm steel column, and a 464 electrochemical detector operated in DC mode at +920 mV). The total run time was 10 min and typically NAS, the last compound in the run, was eluted at 8 min 30 s.

2.4. Histology

The brains were removed from the skulls, kept in 10% formalin for 1 week, and placed in 20% sucrose formalin for 48 h before sectioning. Lesion placement and extents were determined from sliding freezing microtome 30-μm serial cut, stained with thionin. The neural structures and lesion were identified using Paxinos and Watson’s stereotaxic atlas [27].
2.5. Statistical analysis

Results (ng/gland) are expressed as mean ± standard error of the mean (S.E.M.) of the pooled data for each type of experiment, and were computed using GraphPad Prism data analysis and graphing package (V2.0, Graphpad Software, San Diego, California, USA). Comparison between groups were done using the non parametric Mann-Whitney test and, when appropriated, group means were compared to zero using the Wilcoxon signed rank test.

3. Results

All animals were successfully ganglionectomized, since the ganglia were removed under direct microscopic vision. The deep pineal lesion was limited to the aimed region, including the posterior and habenular commissures, the subcommissural organ and, in very few cases, extending to the most dorsomedial part of the precommissural nucleus and the most posterior part of the thalamic paraventricular nucleus (Fig. 1).

All animals sacrificed in the dark had an amount of N-acetylserotonin in the gland significantly different from zero (Table 1), showing an ongoing stimulatory process in the 12–30 h surgically sympathetic deafferented pineal gland.

The nocturnal retinal photostimulation of otherwise intact SCGx rats led to a complete blockage of NAS production (Fig. 2). Concomitantly, there was a significant increase in the content of serotonin, 5-HTP and 5-HIAA.

Fig. 1. Plates A and B show an intact deep pineal/lamina intercalaris region. This is an intercommissural region lying between the habenular and the posterior commissures. C shows a representative lesion of this region. DP, deep pineal/lamina intercalaris region; hbc, habenular commissure; pc, posterior commissure; PCN, precommissural nucleus.
Table 1
Pineal N-acetylserotonin in ganglionectomized rats

<table>
<thead>
<tr>
<th></th>
<th>SCGx Dark</th>
<th>SCGx 15 min light</th>
<th>DP + SCGx Dark</th>
<th>DP + SCGx 15 min light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.E.M.</td>
<td>0.95 ± 0.32</td>
<td>0.06 ± 0.23 a</td>
<td>0.87 ± 0.24 b</td>
<td>0.93 ± 0.28 c</td>
</tr>
<tr>
<td>n</td>
<td>n = 14</td>
<td>n = 14</td>
<td>n = 8</td>
<td>n = 8</td>
</tr>
<tr>
<td>Wilcoxon signed rank test P value</td>
<td>0.001</td>
<td>0.254</td>
<td>0.015</td>
<td>0.035</td>
</tr>
<tr>
<td>Significantly different from zero</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

SCGx, superior cervical ganglionectomized rats; DP + SCGx, deep pineal/lamina intercalaris lesioned, superior cervical ganglionectomized rats.

* Significantly different from SCGx Dark, $P = 0.003$, Mann-Whitney test; a not different from SCGx Dark, $P = 0.247$, Mann-Whitney test; * not different from SCGx Dark, $P = 0.439$, Mann-Whitney test.

There was no difference between the amounts of TRY between the two SCGx groups.

Photic stimulation of DP-lesioned SCGx rats did not produce any change in the quantity of NAS, serotonin, 5-HIAA, 5-HTP or TRY, compared to the respective dark-killed control rats (Fig. 2). In addition, regarding the contents of pineal indolic compounds, there is no statistical significant difference between each one of the DP-lesioned SCGx groups and the dark-killed group of rats that underwent bilateral ganglionectomy alone (Table 1).

4. Discussion

Electrophysiological studies showed that bilateral superior cervical ganglionectomy or decentralization did not prevent the transmission of light signals to the pineal gland either assessed by recording electropinealogram and evoked potentials [8,9,28,31] or extracellular cell unit activity [22]. In addition, it was shown that functional or surgical sympathectomy led to disappearance of the late components of the photic evoked response remaining the early potentials that were abolished after the lesion of the pineal stalk. These data, associated with the well established neuroanatomical demonstration of direct central projections to the pineal gland, lead to the conclusion that light can influence pinealocyte physiology through a neural pathway different from the classical sympathetic peripheral connection.

An open point, however, was the relevance of this central direct neural system to the biosynthetic pineal function. In a previous work we showed [6], for the first time, that DP lesion, i.e., the ablation of the terminal field of all direct central neural connections of the pineal gland, affects the acute photic control of the pineal metabolism. In the present work we tried to directly access the function of those connections making use of the pineal excitatory process elicited by the acute release of noradrenaline in degenerating terminals of the conarii sympathetic nerve fibers after surgical removal of the cell body, situated in the superior cervical ganglion [3,25].

The data showed that light pulses given during the night to ganglionectomized rats is able to completely block NAT activity, reducing NAS and increasing serotonin, 5-HIAA and 5-HTP content of the gland. These observations are indicative of a true shift from the acetylative to
oxidative/deaminative metabolic pathway rather than an artifact due to, e.g., disruption in the pineal blood flow [33] that would either reduce or increase all the pineal products.

However, it has been demonstrated that several types of stress can alter the metabolic capacity of the pineal gland [14,20,21,34,37,39,40,42]. In this way, it is conceivable that the metabolic alterations induced by photostimulation in ganglionectomized rats would be mediated by humoral factors linked to unavoidable stress reaction typical of the experimental situation. Regarding any possible stressful reaction induced by animal handling, the present experiment was adequately controlled since dark and light-killed rats were submitted to the same sort of manipulation. However, the intrinsic difference between the groups, that is, one group was light-stimulated and the other rested in the dark, makes it impossible to discard any nonspecific action of light. Nevertheless, it has been shown [20] that protracted light exposure followed by acute imposition of stress (e.g., physical immobilization) would result in an increase of NAT activity and consequently, pineal NAS production, an effect mediated by adrenal circulating catecholamines, even in sympathectomized rats [21]. If this were the case, the present results would indicate a stronger inhibitory effect of light in SCGx rats.

Other stress-related hormones, such as adrenal corticosteroids, have been shown to reduce NAT activity [14,38,39,43] and might explain the results obtained in the present work. However, for this hormone and any other putative nonspecific inhibitory humoral factor, the DP-leisioned SCGx rats are the more adequate control. The animals of this group were submitted to exactly the same experimental situation (manipulation and light exposure) as the SCGx rats, and did not show any alteration in the pineal indoles induced by light, discounting, consequently, the effect of any humoral factor determining the alteration in the pineal metabolism.

Therefore, we can conclude that in ganglionectomized rats, there is still present a neural mediated photic-induced inhibitory process of the pineal gland whose neuroanatomical substrate depends on the integrity of the deep pineal/lamina intercalaris region.

Such neural-mediated inhibition might be exerted either at pre-synaptic level, blocking the release and/or increasing the reuptake of noradrenaline by the degenerating sympathetic terminals, or at post-synaptic level, blocking the stimulatory action of noradrenaline on pinealocytes. Several putative neurotransmitters are candidates to be the agent of this inhibitory action. It has been shown [30] that GABA, acting on presynaptic receptors are able to decrease the release (GABA_A) and to increase the reuptake (GABA_A) of noradrenaline by the pineal sympathetic terminals. Moreover, it can act on pinealocyte GABA_A receptors, to impair noradrenaline-induced melatonin production [30]. The action of neuropeptide Y on the pineal gland is very similar to GABA. It has been shown [11,26,35,41], depending on the experimental paradigm, that NPY is able to block noradrenaline release by the sympathetic nerve terminals as well as to block its postsynaptic stimulatory effect on pinealocytes, both effects being dependent on pertussis toxin-sensitive inhibitory G protein, albeit mediated by different receptors (NPY-Y2 and NPY-Y1, respectively). Another putative neurotransmitter, acetylcholine, clearly demonstrated as an inhibitory factor on the synthesis of melatonin in bovine pineal gland [29], has, however, contradictory effects on rat pineal gland [10,17,18,32].

Taking together these data from the literature and our previous demonstration [6,7] that the thalamic intergeniculate leaflet and its geniculo-pineal projection pathway are necessary for the full expression of the photo-inhibitory metabolic process induced by nocturnal short-term retinal illumination, the more likely candidate to be the neural substrate of this inhibitory phenomenon is the GABA–neuropeptidergic Y system originating in the IGL. As we stated above, these neurotransmitters are able to block the noradrenergic neurotransmission in the sympathetic terminals as well as to inhibit the ongoing stimulatory postsynaptic activity of noradrenaline on pinealocytes, an action that it would be expected from a system that has to stop acutely the pineal melatonin synthesis and release.

In conclusion, this is, to the best of our knowledge, the first direct demonstration of a neuroendocrine functional role of the central direct neural projections to the rat pineal gland.

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References


