

Effect of chemical denervation with 6-hydroxydopamine on the volume and the nuclear population of the rat pineal gland during the postnatal development

J. Boya, F. Lopez-Munoz and J.L. Calvo

Department of Histology, Faculty of Medicine, University Complutense,
28040 Madrid, Spain

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Abstract

The effect of the chemical denervation with 6-hydroxydopamine (6-OHDA) on the pineal volume and its nuclear population has been studied during the postnatal development of the rat pineal gland. From the 3rd postnatal week onwards, the pineal volume of the animals treated with 6-OHDA is larger than in controls. The number of nuclei of both pineal cell types per unit surface was counted on semithin sections. Mann-Whitney and Kruskal-Wallis non-parametric statistics provided significant differences between the number of both pinealocytes and glial pineal cells in the control and the denervated group at 2 to 4 postnatal months. In both cases, the pineal cell population of the denervated glands was always larger than in control animals. These findings may suggest a delayed maturation of the denervated pineal gland.

Introduction

The pineal gland volume and the size and number of the parenchymal cells may vary according to diverse physiological and experimental conditions. Thus, modifications induced by the photoperiod and the circadian rhythm have been extensively reported (Welsh et al 1979; Dielh 1981; Matsushima et al 1990) as well as those fostered by other natural rhythms, i.e., seasonal (Legait et al 1975; McNulty et al 1980; Kachi and Quay 1984) or estrual (Lincoln 1976; Przybylska et al 1990). Sex is another factor that determine volumetric and structural changes in the pineal gland morphology. Hence, a larger pineal gland volume has been described in females (rat: Legait et al 1976; guinea-pig: Jung and Vollrath 1982). Vollrath (1986) also noted sex-related differences on the pineal gland volume during the first stages of the postnatal development. Recently, we have found a larger number of pinealocytes per surface unit in female

rats during the postpubertal development (Lopez-Munoz et al 1991).

Deep biochemical changes occur in the pineal gland after the surgical denervation by superior cervical ganglionectomy (Wurtman et al 1964; Moore and Rapport 1971; Deguchi and Axelrod 1972; Nagle et al 1973a). Furthermore, ultrastructural changes have been described in the pineal glands of different species after ganglionectomy (Quay 1971; Lin et al 1975; Romijn 1975; Welsh et al 1979; King and Dougherty 1982; Karasek et al 1983; Calvo et al 1990). Nevertheless, owing to technical difficulties, surgical ganglionectomy has been performed only in adult animals, and therefore, the effects of the denervation take place on a gland with a fully developed innervation.

Tranzer and Thoenen (1967) introduced 6-hydroxydopamine (6-OHDA) as a biological tool for denervation. This agent produces a chemical sympathectomy from the first postnatal day, thus hampering the arrival of nerve fibers to the pineal gland. 6-OHDA is a highly selective agent for the destruction of noradrenergic neurons (Jonsson 1971) and without toxic effects on other cells of the nervous system (Bloom et al 1969). Its administration to newborn animals (Angeletti and Levi-Montalcini 1970; Angeletti 1971), enhances the neurotoxic effects, eliciting an "irreversible chemical sympathectomy", in contrast to the reversible denervation that occurs when applied to adult animals (Thoenen 1971).

The aim of the present work is to study volumetric changes in the pineal gland, and to analyze the modifications of the nuclear population during the postnatal development of 6-OHDA chemically denervated neonatal rats.

Materials and Methods

Fifty-six Wistar rats kept under routine lighting (14L:10D) and feeding conditions, were sacrificed at the following ages: 1, 3, 5, 15 days, 1, 2, 3 and 4 months. An equal number of males and females were used in each stage.

Every litter was divided in two experimental groups for further treatment; a control group and a denervated group. Chemical sympathectomy was performed by daily administration of 6-OHDA throughout the first 5 postnatal days (Angeletti 1971). A dose of 50 mg of 6-OHDA (6-hydroxydopamine hydrochloride, Fluka, Switzerland) per kg of body weight in 0.9% saline supplemented with 0.5 mg ascorbic acid per ml of solution, prepared immediately before the administration, was injected subcutaneously. Control animals were injected with an equal volume of solvent during the same period.

To avoid morphological variations due to chronobiological rhythms, all animals were killed by decapitation under ether anesthesia, at 18:00 h during the months of April and May. For assessing the pineal volume, a male and a female were sacrificed per experimental group and age interval. Pineal glands were fixed by immersion in Bouin's solution, embedded in paraffin, serially cut at 7 μm thick and stained with hematoxylin-eosin.

Glandular volume was estimated by the method proposed by Vollrath (1986). The surface of every fourth serial tissue sections was measured. Subsequently, the mean surface of two consecutive measured tissue sections was assessed and this figure multiplied by the tissue section thickness (4x7 μm). The addition of all volumes

obtained corresponded to the total gland volume.

To establish the hypothetical differences in the pineal nuclear population, four pineal glands obtained from two males and two females, were taken for each experimental group within the last three age intervals studied (2, 3 and 4 months). These pineal glands were fixed by immersion in 2% glutaraldehyde - 2% paraformaldehyde in 0.1 mol/l phosphate buffer pH 7.4 at 4°C. After fixation, tissue blocks were washed in 0.1 mol/l phosphate buffer, postfixed in 1% osmium tetroxide in the same buffer and embedded in Vestopal. Semithin sections 0.5 μm thick were cut with a LKB ultramicrotome and stained with the silver-ammonium impregnation method according to Klein et al (1981).

Five different areas of the semithin sections, including peripheral and central areas, amounting 26,377 μm^2 per gland, were used to assess the number of nuclei of the two pineal cell types. Surfaces with large areas of connective tissue were rejected to avoid intraglandular topographic variations described in the rat pineal gland (Dieth 1981; Heidbuchel and Vollrath 1983). A computerized image analysis system (VIDS IV^R) was used for the nuclear count and to measure the surface of the aniline stained sections.

The statistical analysis of the data was accomplished by the Mann-Whitney and the Kruskal-Wallis non-parametric tests. Numerical data were expressed as the mean number of nuclei \pm standard error of the mean (SEM).

Results

The results of the present study are shown in figure 1 and tables I-III. Figure 1 shows the volumetric evolution of the denervated pineal gland as compared to the control. Data are expressed as the mean of the volumes obtained from males and females in each experimental group (Table I). The volume of 1-day-old control pineal glands averages 0.055 mm^3 . The volumetric growth is fast during the first two postnatal weeks, when the volume reaches six times that of the first day. Since then, the enlargement is much less steep, whereby at the forth month of postnatal life, the volume (0.641 mm^3) is only twice that at the 15 postnatal day (0.289 mm^3).

The curve of the pineal volume of 6-OHDA treated rats differs from that obtained in the control group (Fig. 1). Thus, until the third postnatal day, the volume of the gland is similar in the control and denervated groups. From this stage, and up to the end of the third postnatal week, volumes are lower compared to those of control pineal glands. However, the volume of the denervated glands is larger from here to the end of the period studied. In the group of sympathectomized animals, the peak of rapid growth is reached at the 30 postnatal day (0.399 mm^3) when the gland presents a similar volume to that of 2-months-old control rats (0.405 mm^3). In the last stage studied (4 months), the pineal volume of the group treated with 6-OHDA continues to be larger than the control, though tending to equate.

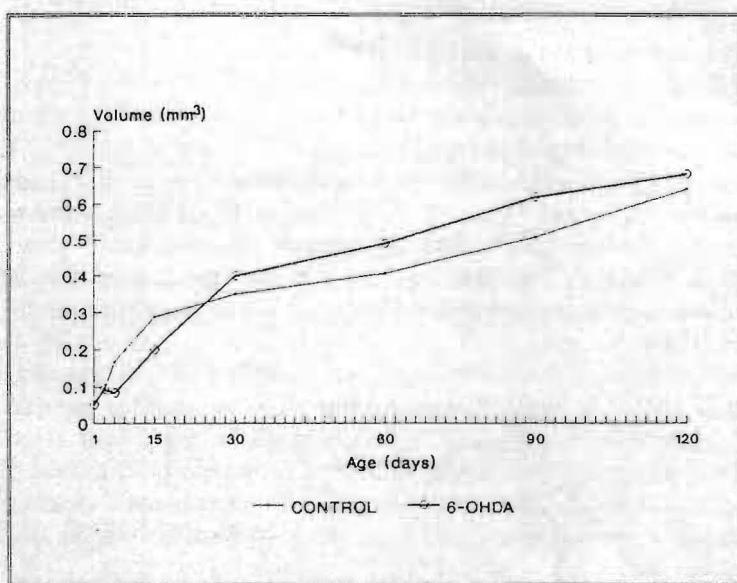


Figure 1: Comparative evolution of the rat pineal gland volume along the postnatal development between control and 6-OHDA treated rats.

Table I: Volume of the pineal gland in control and 6-OHDA treated rats during postnatal development*.

Age (days)	Control	6-OHDA
1	0.055	0.051
3	0.095	0.094
5	0.168	0.083
15	0.289	0.199
30	0.350	0.399
60	0.405	0.489
90	0.506	0.617
120	0.641	0.681

*Pineal gland volume in mm³.

Table II: Number of pinealocyte nuclei per surface unit in control and 6-OHDA treated rats. Morphometric analysis.

Age Months	Control			6-OHDA			H*	
	Male	Female	U	Male	Female	U		
2	171.9±5.9	251.1±6.8	p<0.05	262.0±2.8	264.9±4.1	N.S.		
	M	219.4±10.0			263.4±2.4			p<0.01
3	182.0±6.7	230.8±3.8	P<0.05	235.0±5.1	239.5±3.6	N.S.		
	M	221.3±6.5			237.2±3.1			p<0.01
4	164.0±5.1	220.7±7.6	p<0.05	210.0±4.6	236.5±4.8	p<0.05		
	M	207.7±9.4			223.2±4.7			N.S.
H**	p<0.001							

*Values are mean ± S.E.M. for each age and sex. Area = 26,377 μm^2

M = mean number of pinealocyte nuclei ± S.E.M. for both sexes in each age interval.

U = Level of significance for age interval according to the Mann-Whitney U-test. N.S. = not significant.

H* = Level of significance for both experimental groups in each age interval according to the Kruskal-Wallis H-test. N.S. = not significant.

H** = overall level of significance for all age intervals according to the Kruskal-Wallis H-test.

Tables II and III show mean values ± SEM obtained from the count of the nuclei of pinealocytes and glial pineal cells per surface unit ($26,377 \mu\text{m}^2$). Table II shows results for the main parenchymal pineal cell. The Mann-Whitney U-test revealed statistically significant sex-related differences ($p<0.05$) for the control group in all age intervals studied. In the denervated group, no statistically significant sex-related differences were noted, except for the 4 months stage. The comparison between the two groups through the Kruskal-Wallis H-test evidences significant differences in the number of pinealocytes per surface unit in the intervals of 2 and 3 months ($p<0.01$), while for the 4 months interval differences were not significant. On the other hand, the mean number of nuclei in 4-months-old denervated pineal glands (223.2 ± 4.7) is similar than the mean for 2-months-old control pineal glands (219.4 ± 10.0).

No statistically significant sex-related differences were observed in glial pineal cells, supporting previous results (Lopez-Munoz et al 1991). Thus, values of Table III are the mean of those obtained in males and females for each interval and group. On the contrary, the Mann-Whitney U-test revealed a striking difference in the number of glial

Table III: Number of glial cell nuclei per surface unit in control and 6-OHDA treated rats. Morphometric analysis*.

Age (months)	Control	6-OHDA	U
2	21.95 ± 0.85	36.87 ± 0.99	p < 0.001
3	17.95 ± 0.78	32.25 ± 0.96	p < 0.001
4	20.50 ± 1.56	25.44 ± 0.99	p < 0.01
H	p < 0.01		

*Values are mean ± S.E.M. for each age. Area = 26,377 μm^2

U = level of significance for age interval according to the Mann-Whitney U-test.

H = overall level of significance for all age intervals according to the Kruskal-Wallis H-test.

pineal cells between the two experimental groups (Table III). Thus, as with pinealocytes, the number of glial cell nuclei per surface unit is always larger in animals treated with 6-OHDA, whereby at 2 and 3 postnatal months their number almost doubles that of control animals ($p < 0.001$), while in 4-months-old rats only a 25% increase was found ($p < 0.01$).

Discussion

The effects of the surgical sympathectomy on the pineal gland of adult animals have been extensively studied. The atrophy and the hypofunction of pinealocytes after the denervation is well known (Welsh et al 1979; Karasek et al 1983; Calvo et al 1990). Furthermore, a striking decrease in the biochemical pineal contents has been described after the surgical denervation (Wurtman et al 1964; Klein et al 1971; Moore and Rapport 1971; Deguchi and Axelrod 1972; Nagle et al 1973a). In contrast, Quay (1971) found no differences on the adult rat pineal volume after the superior cervical ganglionectomy.

The chemical denervation using 6-OHDA has also evidenced pineal modifications both at the ultrastructural (Sheridan and Keppel 1971; Romijn 1976; Reuss 1989) and the biochemical level (Illnerova 1975; Lynch et al 1977; Shivers et al 1979; Reuss and Oxenkrug 1989), which are similar to those observed after surgical sympathectomy.

The volume of the 6-OHDA denervated pineal gland is larger than the control from the 3rd postnatal week. The total volumetric increase from the first postnatal day to the fourth month is also larger in denervated pineal glands (13.35 times) respect to controls, whose volume multiplies only by 11.6. Vollrath (1986) only found a 4-fold

increase between newborn and adult Sprague-Dawley rats.

Denervated rats exhibited a larger pineal volume and a higher cell population per surface unit (24.5% more at the 2nd postnatal month, 12.5% at the 3rd month and 8.8% at the 4th month). Such findings indicate that the effect on the pineal volume is secondary to hyperplasia of pineal cells and not to cell hypertrophy. This may suggest a delay in the glandular maturation of 6-OHDA treated rats, i.e., a sustained and/or increased postnatal cellular proliferation.

On the other hand, sex-related differences in the number of pinealocytes per surface unit in normal rats (Lopez-Munoz et al 1991), are neglected in denervated animals, except the 4-months-old interval ($p < 0.05$, Table II). The surgical denervation has evidenced a decrease in the estradiol levels and a lack of response in the pineal gland both to estrogens (Nagle et al 1973b; Cardinali 1977) and to testosterone (Nagle et al 1975). This could explain the sex-related similarities that we found in the cell population of the chemically denervated group.

According to our findings, glial cells, second pineal parenchymal cell type, showed no statistically significant sex-related differences. Nevertheless, notable differences were noted in both experimental groups regarding the number of glial pineal cells per surface unit. Calvo et al (1990) have also described a slight increase of this cell type in surgically sympathectomized adult rats. These findings support the close relationship between this cell type and the pineal innervation.

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Address for correspondence:

Prof. J. Boya
Department of Histology
Faculty of Medicine
University Complutense
28040 Madrid, Spain