Effects of sham-pinealectomy, performed under white and red light, on the melatonin content of rat pineal glands

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Summary. Sham-pinealectomy, performed under different light conditions in newborn and adult rats, is followed by changes of pineal activity resulting in variations of melatonin content. The pineal glands of rats sham-operated under white light produce significantly less melatonin. In contrast, glands of rats operated on under red light show a melatonin content corresponding to that of intact rats. This result implies that normal white light causes a disturbance in melatonin production by a non-retinal pathway.

In multiple earlier experiments performed in order to study tumor development in pinealectomized (PiX) rats we observed a difference of response between control groups of intact animals without any surgery and sham-operated rats. These effects occurred almost constantly[1].

Assuming that sham-surgery itself might cause some changes in the melatonin content of the pineal - due to direct influence of light on the pineal body in situ - we measured the content of melatonin in the pineal glands of rats subjected to sham-surgery under varying light conditions.

Materials and methods. Male Wistar rats were housed under standard conditions and a light/dark 12/12 h lighting schedule. Randomized rats from newborn litters were subjected to sham-pinealectomy[1] under white and red light, respectively.

The experimental groups were: group A, I: sham-pinealectomy performed in newborn rats (aged 24-48 h) under white light. II: sham-pinealectomy carried out in newborn rats under red light; group B, I: sham-pinealectomy performed in adult rats (35 days old) under white light. II: sham-pinealectomy carried out in adult rats under red light; group C: normal intact rats without any surgery subjected to anesthesia only. Light sources used were: white light lamp of 75 W, red light, ruby lamp Philips PF 712 E/6H.

At the age of 2 months all animals were killed 3.5 h after onset of darkness. Each pineal gland was quickly removed and stored in liquid nitrogen. Melatonin was measured fluorimetrically according to the method of Suzuki[2]. The concentration of OPT, the fluorophor-forming agent, was used as described by Tachiki[3]. The weight of each gland was precisely determined, 2 glands were pooled for each analysis, and the amount of melatonin was expressed in µg/g pineal tissue.

Results. As can be seen from the table, highly significant differences in the melatonin content of the pineals were obtained between the groups of rats operated on under white and red light, respectively. The amounts of melatonin determined in the 2 groups operated on under red light (AII and BII) differ slightly from the melatonin values of the control group (rats without surgery). However, pineal melatonin or rats operated on under normal light (groups AI and BI) was reduced to approximately 50% in comparison with the intact control group.

Melatonin content of rat pineal glands in µg/g wet wt ± SD

	A) Sham-PiX neonatal	B) Sham-PiX adult	C) Control
I. White light	0.216 ± 0.011* (n = 5)	0.287 ± 0.055* (n = 6)	
II. Red light	0.593 ± 0.032** (n = 7)	0.459 ± 0.027*** (n = 5)	0.547 ± 0.030 (n = 6)

Number of rats in parenthesis. Statistical analysis: Student's t-test: * Different from the control group $p < 0.002$; ** no significant difference from the control group; *** different from the control group $p < 0.05$.

Discussion. The biosynthesis of serotonin and melatonin in the pineal gland shows light-dependent circadian rhythms, starting from the 6th postnatal day. This coincides with the beginning of serotonin production. The circadian rhythms are fully developed in 35-day-old rats, when hydroxy-indol-O-methyltransferase (HIOMT) has reached its full activity[4]. In lower vertebrates, light has a direct effect on the pinealocytes, whereas in mammals the effects are mediated by retinal and neural pathways.

The observation that the reactivity of sham-pinealectomized rats differs from that of intact rats without surgery has been reported by other authors[6,7] and confirmed by our experiments[1]. We therefore supposed that the endocrine activity of this gland might be directly affected by white light during sham-extirpation. Our experiment demonstrates that melatonin biosynthesis is altered by intensive white light hitting the pineal in situ during sham-operation, but it is left unaltered when surgery is performed under red light.

In order to explain these results, 2 aspects have to be considered: The pineal gland contains 2 light-sensitive enzymes, N-acetyltransferase (NAT) and hydroxy-indole-O-methyl-transferase (HIOMT) which are both involved in the biosynthesis of serotonin to melatonin. This sensitivity is mediated reversibly by the retinal pathway[8,9]. As HIOMT matures only postpubertally, this enzyme cannot be responsible for the reduced melatonin production of group AI. However, in our experiment the melatonin synthesis was reduced irreversibly for at least 2 months.

The pineal gland contains extremely photosensitive pteridine-like substances[10]. Tetrahydrobiopterin acts as a coenzyme of tryptohydroxylase during serotonin synthesis. Degradation of this substance can reduce the production of serotonin followed by a lower melatonin level in the pineal body. Whether the serotonin level in the pineal of sham-pinealectomized rats is affected by light, will be clarified in our next experiments.

According to our results it is necessary to emphasize that, for experiments in pinealectomized rats, the use of control animals with sham-surgery carried out under normal light is no equivalent for a control group of intact animals without any surgery, as far as pineal melatonin production is concerned.

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