Acute effects of unilateral or bilateral superior cervical ganglionectomy on rat pineal N-acetyltransferase activity and melatonin content[1]

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Summary. Acute bilateral superior cervical ganglionectomy (SCGX) completely prevents the nocturnal rises in pineal N-acetyltransferase (NAT) activity and melatonin content in male rats kept in light-dark cycles of 14:10. Unilateral SCGX causes the NAT and melatonin levels to be intermediate between those in sham-operated control rats and those in rats from which both ganglia had been removed.

Within the pineal gland, serotonin is N-acetylated by the enzyme serotonin N-acetyltransferase (NAT) to form N-acetylserotonin[2]. The latter compound is then O-methylated in the presence of enzyme hydroxyindole-O-methyltransferase to form melatonin[3]. NAT is believed to be rate limiting in melatonin synthesis[4].

When animals are kept under alternating periods of light and darkness (LD cycles), the conversion of serotonin to melatonin increases greatly during the dark period. In the case of the rat, pineal NAT activity is increased 30–70-fold during the night[5] whereas the pineal content of melatonin rises roughly 10-fold compared to daytime levels[6].

The sympathetic nervous system plays a vital role in determining the biosynthetic and secretory activity of the pineal gland[7]. The postganglionic fibres which innervate the pineal have their cell bodies in the superior cervical ganglia[8]. The present study was designed to investigate the effects of either acute unilateral or bilateral superior cervical ganglionectomy on the nighttime rises in pineal NAT activity and melatonin content.

Materials and methods. Experiment 1. 120 male Sprague-Dawley rats weighing 75–100 g were acclimated for 1 week to a LD14:10 photoperiod. Lights were automatically turned off daily at 20.00 h. After the 1-week period of acclimation, 40 rats were bilaterally superior cervical ganglionectomized (SCGX), 40 were unilaterally SCGX, and 40 were sham-operated (intact controls). The animals were returned to the LD14:10 photoperiod following the operations. On the second day after the surgical procedures, subgroups of 6–7 animals (of each of the 3 groups of rats) were killed at 4-h intervals throughout a 24-h period. Pineal glands were recovered and frozen on solid CO_2 until they could be assayed. When animals were killed during the period of darkness a dim red light aided in the recovery of the pineal gland. Pineal NAT activity was estimated by a modification of the method of Deguchi and Axelrod[9] as previously described[10]. The results are expressed as nmoles ^{14}C-N-acetyltryptamine synthesized per pineal per h. Statistical analysis was performed on a Monroe programmable calculator using an analysis of variance followed by a test for differences between means and the Student t-test.

Experiment 2. 120 male rats from the same supplier mentioned above were housed as in experiment 1; the surgical procedures were also the same. On the second day following the operations (sham, unilateral or bilateral SCGX), subgroups of rats were again killed at 4-h intervals over a 24-h period. Pineal glands were quickly dissected and frozen on solid CO^2. Pineal melatonin content was measured using the radioimmunoassay procedure described by Rollag and Niswender[11]. The data were analyzed using the same statistical procedures described above.

Results. Experiment 1. In intact control rats, daytime levels of NAT activity were low (figure 1). Within 4 h after lights out (24.00 h) NAT activity increased 31-fold ($p < 0.001$ vs day values); at 04.00 h (8 h after darkness) the NAT activity had increased to its highest level (58 times higher than day

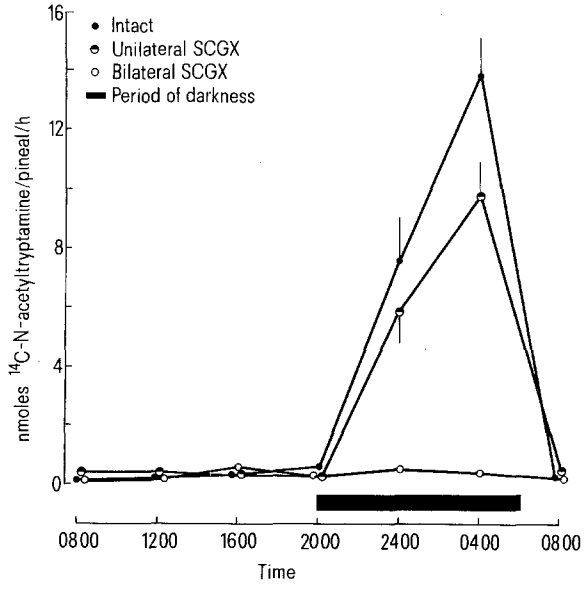

Fig. 1. Effect of unilateral or bilateral superior cervical ganglionectomy (SCGX) on pineal serotonin N-acetyltransferase activity in male rats. Each point represents the mean value for 6 or 7 rats. Vertical lines from points represent SE. On points where SE are missing the errors were either so small as to be obliterated by the point or they would have unnecessarily confounded the figure.

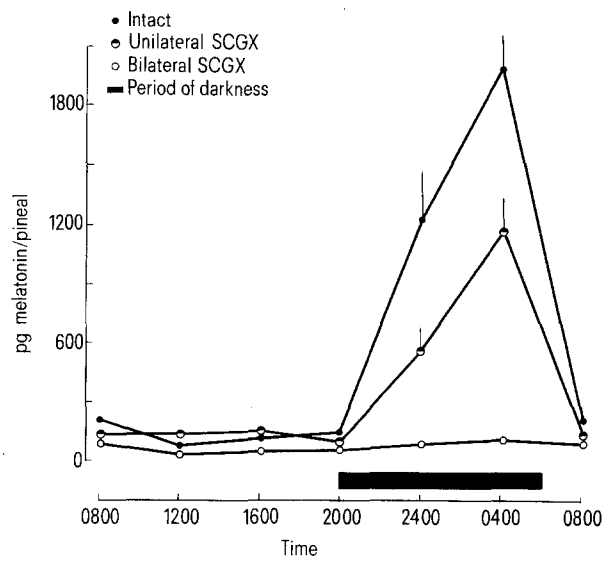

Fig. 2. Effect of unilateral or bilateral superior cervical ganglionectomy (SCGX) on pineal melatonin levels in male rats. Each point represents the mean value for 6 or 7 rats. Vertical lines from points represent SE. On points where SE are missing the errors were either so small as to be obliterated by the point or they would have unnecessarily confounded the figure.

levels; $p < 0.001$ vs day values). By 2 h after the onset of light, pineal NAT activity in intact rats had returned to levels normally measured during periods of light. Bilateral SCGX completely abolished the nighttime rise in pineal NAT activity (figure 1). Unilateral SCGX rats had nighttime NAT values intermediate between those of intact or bilaterally SCGX rats. Pineal NAT activity in unilaterally SCGX rats increased to 20 and 37 times daytime values at 24.00 and 04.00 h, respectively. The 04.00 value in the unilaterally SCGX rats was lower ($p < 0.02$) than that in the intact rats and higher ($p < 0.001$) than that of rats lacking both their superior cervical ganglia.

Experiment 2. As with the NAT levels, pineal melatonin content in intact rats rose dramatically during the night; at 24.00 h the melatonin content was 9.5 times greater than daytime values while at 04.00 h this increased to 15.4 times higher than the mean values during the period of light (figure 2). Removal of both superior cervical ganglia completely prevented the nocturnal rise in melatonin while unilateral SCGX caused the nighttime values of melatonin to be at an intermediate level. Hence, at both 24.00 and 04.00 h the pineal melatonin content of the unilaterally SCGX rats was significantly lower ($p < 0.001$) than that of intact animals but significantly higher ($p < 0.001$) than similar values in the bilaterally SCGX rats.

Discussion. The nocturnal rise in pineal NAT activity in sham-operated rats reported in this study is comparable to that observed in similar experiments by other workers[5,7,12]. Usually the magnitude of the increase is on the order of 30–70-fold; in the present experiment NAT activity was 58 times greater at 04.00 h (during darkness) than during the day. The rise in pineal melatonin content during darkness also has been reported previously[6,13]. A strong correlation has been shown to exist between the nighttime rise in NAT activity and the increased content of melatonin[6]. Although in the present study NAT and melatonin were not assayed in the same glands, the observed results support a relationship between NAT activity and melatonin production.

Sympathetic denervation of the pineal gland (by removal of the superior cervical ganglia) has repeatedly been shown to negate the ability of the pineal to influence other endocrine organs[14,15]. As observed here, acute bilateral SCGX also is associated with an absence of a nocturnal rise in either NAT activity or melatonin during the second night following the operation. This is likely due to the loss of the neurotransmitter norepinephrine from the nerve endings within the pineal gland which follows interruption of the postganglionic sympathetic fibres to the pineal[16]. Unilateral SCGX curtailed the nighttime rises in both NAT activity and melatonin content. However, in contrast to the effects of bilateral SCGX, the increases in these parameters were not completely prevented by unilateral SCGX. It appears that unilateral SCGX impairs the ability of only a portion of the pinealocytes, the supposed functional elements of the pineal, to respond to darkness. It has not been determined whether long-term unilateral SCGX would also reduce, by an equivalent proportion, the ability of the pineal to suppress endocrine functions.

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The involvement of serotonin in induced ovulation in the immature rat

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Summary. In pregnant mare's serum gonadotropin (PMS) treated immature rats the cortex, cerebellum, caudate nucleus and hypothalamus were isolated and analyzed for their serotonin (5-HT) content at 6-h intervals for 72 h. Results showed a general trend of significant variation occurring in days 1 and 3 after PMS injection with no major variations observed on the second day. The results obtained suggest a possible involvement of 5-HT in the control of ovulation.

A considerable body of literature supports the concept of serotoninergic interference with ovulation in rats[2–5]. Pregnant mare's serum gonadotropin (PMS) treated immature female rats given varied doses of serotonin (5-hydroxytryptamine, 5-HT) have been shown to produce a much smaller number of ova when compared with those recovered from control rats[2].

Other studies associated this interference with an inhibitory effort of 5-HT on certain hypothalamic catecholaminergic neurons that are normally responsible for facilitating ovulation[6–8]. Although many attempts have been made to correlate this inhibition on ovarian activity with endogenous 5-HT, few if any investigators have observed actual levels of the compound in the brain during the period leading up to ovulation. It was of interest, therefore, to examine 5-HT levels in the brain at different intervals during the initial ovulatory period in immature rats.

Methods. 24-day-old female rats of the Sprague-Dawley strain (Southern Animal Farm, Alabama), weighing 50–70 g, were maintained at a constant temperature (22±1 °C) in a light-controlled room (light on from 09.00 to 21.00 h). Animals were provided with standard Purina Lab Chow and water ad libitum. After a period of 4 days, 36 rats were injected s.c. with saline solution containing 25 IU PMS