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Pineal Dependence of the Syrian Hamster's Nocturnal Serum Melatonin Surge

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The usual nocturnal surge of pineal melatonin content was blocked by bilateral superior cervical ganglionectomy in male Syrian hamsters. Ganglionectomy and pinealectomy each prevented the nocturnal rise of serum melatonin concentration seen in control animals. The normal nocturnal surge of circulating melatonin in this species appears to depend on the pineal gland and its sympathetic innervation.

Key words: pineal, melatonin, Syrian hamster, sympathetic, ganglionectomy

INTRODUCTION

The Syrian hamster is the species most often used to demonstrate the effects of the pineal gland on the reproductive system and thyroid function [Reiter, 1980; M. Vaughan et al., 1982; M.K. Vaughan et al., 1982; Vriend, 1983]. Previous pineal melatonin content profiles [Panke et al., 1979; Tamarkin et al., 1979] and the more recently studied serum concentration profiles [Brown et al. 1981; Vaughan et al., 1985a] both are characterized by a nocturnal melatonin surge in the late part of the dark phase in this species. It is often assumed that the hamster pineal gland is the major source of the nocturnal rise in circulating melatonin concentration. The present study tests this hypothesis.

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MATERIALS AND METHODS

Young adult male hamsters (*Mesocricetus auratus*) were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) and maintained in our facilities in a light/dark (L/D) cycle of 14/10 h (light on 0600 h) until sacrifice. After a month in the L/D cycle, the animals were divided into three groups based on surgical procedures: sham pinealectomy (CON); bilateral superior cervical ganglionectomy (SCGx), verified by presence of postoperative ptosis bilaterally in each case; and pinealectomy (Px), verified grossly at autopsy in each case. Three weeks after surgery, six to seven members of each group were decapitated by guillotine (alternating among the groups) at 2000 h in the light or at 0200, 0400, or 0600 h in the dark under dim red light (two 25-W incandescent globes, each with a Kodak No. 1A filter). Pineals and trunk serum were frozen for later determination of melatonin using the Rollag antibody [Vaughan et al., 1985a,b]. This procedure includes acid and alkaline washes of the $CHCl_3$ sample extract and petroleum ether washes of the reconstituted aqueous phase, has coefficients of variation within assay of 10% (31–35 pg/ml) and between assay of 11% (57 pg/ml), yields a least detectable concentration (250 μl sample size) of 10 pg/ml, and results in hamster serum values similar to those obtained in other studies with another antibody [Brown et al., 1981]. Differences among group means were assessed using a t test with t values adjusted for inequality of variance and of sample size between groups in a comparison and with P values adjusted to account for multiplicity of comparisons by the Bonferroni method [Dixon, 1983].

RESULTS

Figure 1 shows that the pineal melatonin content of SCGx animals was significantly lower than in CON at 0200 h, 0400 h, and 0600 h. No significant difference between the 2000 h SCGx mean and any other SCGx mean was detected. Figure 1 also shows that the nocturnal rise in serum melatonin concentration seen in CON was not present in the SCGx or in the Px animals. The difference of means between CON and each of the other two groups was significant at 0400 and 0600 h. The 2000 h means for the SCGx and the Px groups were not significantly different from any of the other respective means for those surgical groups. No difference among groups was detected at 2000 h, or between SCGx and Px at any time. All values were in the detectable range (above 10 pg per ml serum or per pineal).

DISCUSSION

Blocking the normal nocturnal rise of meiatonin content within the hamster pineal gland by SCGx confirms a previous report [Panke et al., 1979] and fits the general mammalian model in which the pineal's sympathetic innervation mediates the gland's nocturnal rise in enzymatic synthesis of melatonin [Reiter et al., 1975; Moore, 1978; Reiter, 1984; Vaughan, 1984]. We now show that both denervation and removal of the pineal gland block

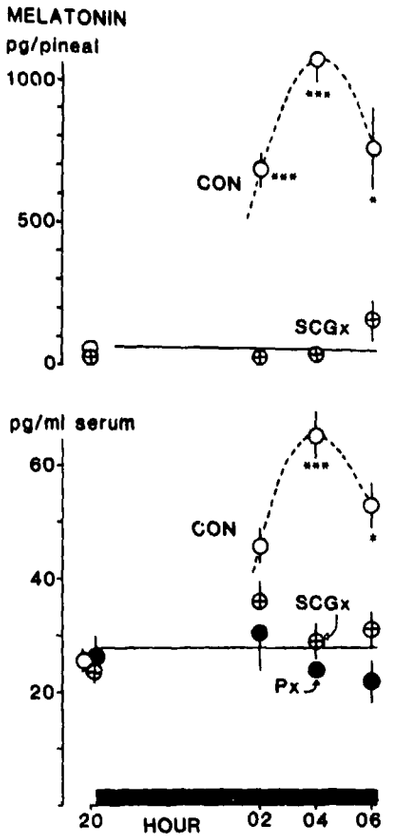

Fig. 1. Pineal (upper panel) and serum (lower panel) melatonin values (mean ± SE) in Syrian hamsters. The solid abscissal bar indicates the time of darkness, which does not include the 2000 h but does include the 0600 h samples. SCGx, superior cervical ganglionectomy; Px, pinealectomy; CON, sham-Px controls. $^{*}P < 0.05$ and $^{***}P < 0.001$ vs any other group at that time.

the late nocturnal rise in serum melatonin, demonstrating dependence of the usual nocturnal surge in circulating melatonin not only on the pineal but also on its sympathetic innervation. Dependence of the nocturnal blood [Vaughan et al., 1979] and urinary [Kneisley et al., 1978; Tetsuo et al., 1981] melatonin surge on an intact central and peripheral sympathetic nervous pathway in human beings also has been shown previously, as well as loss of detectable serum melatonin in a patient after removal of a tumorous pineal [Neuwelt and Lewy, 1983]. Those data and the present results together strongly suggest that a neurally dependent rise in pineal melatonin secretion accounts for the normal nocturnal elevation of blood melatonin concentration in humans and hamsters.

Experiments in other species that normally exhibit a nightly rise in circulating melatonin have not included ganglionectomy, but are in agreement with these results. The nocturnal rise in immunoassayable plasma melatonin did not occur in Px rats [Ozaki and Lynch, 1976]. Blood melatonin

was undetectable even at night after Px in chickens [bioassay, Pelham, 1975] and rats [bioassay, Pang and Ralph, 1975; mass spectral assay, Lewy et al., 1980]. In the above studies, nocturnal blood was sampled at only one time point in Px rats. Pinealectomized sheep had no nocturnal rise of immunoassayable blood melatonin [Arendt et al., 1980; Kennaway et al., 1977, 1982; Bittman et al., 1983].

Pinealectomy of rats blunted or eliminated the rise of urinary melatonin excretion at night [bioassay and radioimmunoassay, Ozaki and Lynch, 1976]. The 24-h urinary excretion (not partitioned into night and day samples) of the major melatonin excretory product 6-hydroxymelatonin (mass spectral assay) was reduced by more than 95% after Px in rats [Markey and Buell, 1982] and monkeys [Tetsuo et al., 1982]. The above findings suggest that potential extrapineal sources of melatonin, such as the digestive organs, retina, and Harderian gland [Quay and Ma, 1976; Cremer-Bartels, 1978; Brammer et al., 1978; Bubenik et al., 1978; Bubenik, 1980; Bubenik, 1981; Ralph, 1981; Pevet et al., 1981; Reiter et al., 1983], may not account for much of circulating melatonin after Px. Of course, the contribution of such other sources in mammals has not been fully tested, in that circulating and urinary measurements have not been made after removal of only those sources.

The present study does not address the issue of the origin of the persistent melatonin detectability after SCGx and Px in these hamsters and after Px in rats [Ozaki and Lynch, 1976] and sheep [Kennaway et al., 1977; Bittman et al., 1983]. Possibilities include nonspecificity of radioimmunoassay antibodies as well as the alternate sources of melatonin mentioned above. Experimental assessment definitively distinguishing between these possibilities is not available in hamsters and sheep. The composite published results in rats suggest antibody nonspecificity as a likely possibility in that species, and such a phenomenon might conceivably contribute to apparent melatonin detectability during the day and at night after Px and SCGx in our hamsters.

Nevertheless, in our study of hamsters, pineal melatonin content did not rise in SCGx at the expected time as seen in controls, the lower serum values were similar between SCGx and Px in the dark phase at the time of the nocturnal surge seen in the serum of controls, and the dark phase serum values of SCGx and Px were similar to the late light phase (2000 h) values for all groups. These observations strongly suggest that the normal nocturnal surge of circulating melatonin concentration in hamsters depends on both the pineal gland and its sympathetic innervation. Whether the apparent melatonin levels seen after Px and SCGx have an extrapineal source or include nonspecific cross-reactivity and whether surges of melatonin might occur in Px or SCGx hamsters at some other time in the 24-h cycle remain to be investigated.

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