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Day–Night Differences in the Response of the Pineal Gland to Swimming Stress[1] (42670)

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Abstract. The effect of swimming stress on pineal *N*-acetyltransferase activity, hydroxyindole-*O*-methyltransferase (HIOMT) activity, and melatonin content was studied during the day and night in adult male rats. At night, elevated pineal activity was suppressed by light exposure before the animals swam. During the day, swimming for 2 hr did not stimulate NAT activity unless the animals were pretreated with desmethylimipramine (DMI), a norepinephrine uptake blocker. Pineal melatonin content after daytime swimming exhibited a weak rise, unless DMI was injected, in which case melatonin levels showed a highly significant increase. Swimming at night caused a greater (compared to daytime levels) increase in NAT activity in both noninjected and DMI-injected rats. Melatonin levels at night were highly significantly stimulated (compared to daytime values) even without pretreatment of the rats with DMI. The greater response of the rat pineal to swimming stress at night may relate either to an increase in the number of β-adrenergic receptors in the pinealocyte membrane at night or to a reduced capacity of the sympathetic neurons in the pineal to take up excess circulating catecholamines. Pineal HIOMT activity was not influenced by swimming (with or without DMI) either during the day or at night.

In the rat, the nocturnal rise in pineal *N*-acetyltransferase (NAT) activity and melatonin levels is regulated by an increase in the synthesis and release of norepinephrine (NE) from intrapineal postganglionic sympathetic nerve terminals (1, 2) and by a rise in the number of β-adrenergic receptors in the pinealocyte membrane (3, 4). NE interacts with the β-receptors and initiates a cascade of events which involves the second messenger, cyclic AMP, and which eventually results in a rise in the quantity of melatonin formed (5). Since stressful stimuli also release NE from a variety of organs, most notably the adrenal medullae, and the catecholamine reaches the pineal gland via the systemic circulation where it could promote melatonin production, a number of studies have examined the influence of aversive demands on the activity of pineal NAT and the melatonin content of the gland (6–9). All of these studies were conducted during the day when pineal NAT activity and melatonin content are low and when the number of pinealocyte β-receptors available for stimulation by NE is likewise low (3, 4). Although some of the studies showed that low daytime melatonin levels could be stimulated (6) by stressful stimuli which increased circulating catecholamines (10), these rises are believed to be blunted by virtue of the fact that the sympathetic nerve endings in the pineal gland actively take up circulating catecholamines thereby protecting the pinealocytes from stimulation. This contention is based on the observation that the administration of a NE uptake inhibitor, e.g., imipramine or desmethylimipramine, greatly exaggerates the pineal NAT response to stress (11).

Since the stress experiments have been conducted primarily during the day when the number of pinealocyte β-receptors is depressed, we decided to compare the response of the rat pineal gland to an aversive stimulus during both the day and the night when the number of β-receptors on the pinealocyte is lowest and highest (3, 4), respectively. Thus, the purpose of the current study was to determine whether the increased number of

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β-adrenergic receptors in the rat pinealocyte membrane was significant in terms of the physiological response of the gland to aversive demands. The stress used in this study was swimming in 30°C water and the endpoints include pineal NAT activity, hydroxyindole-*O*-methyltransferase activity (HIOMT), the melatonin-forming enzyme, and radioimmunoassayable melatonin levels.

Materials and Methods. Adult male Sprague–Dawley rats, weighing 180–200 g, were purchased from Harlan–Sprague–Dawley, Houston, Texas. Animals were housed four per polycarbonate cage and were given food and water *ad libitum.* They were exposed to a light:dark (LD) environment of 14:10 (lights on 0600–2000 hr daily) and to a regulated ambient temperature (22 ± 2°C).

For the first half of the study, 21 rats were divided into three equal-sized groups. Seven of these were given a subcutaneous injection of desmethylimipramine (DMI, 10 mg/kg body wt), a NE uptake inhibitor, in 0.1 ml saline at 1350 hr. Beginning at 1400 hr, these 7 animals plus 7 otherwise untreated rats were placed in a tank containing 30°C water. The depth of the water was such that the animals could not easily touch the bottom unless their head was submerged; also, because of the smooth surfaces the animals could not hold on to the sides of the container and, as a result, they swam virtually continually during the experimental period. After 2 hr, at 1600 hr the animals were removed from the water and their pineal glands were collected. At the same time the pineal glands of 7 control rats that had not swam were also collected. Pineals were frozen on solid CO_2 and stored at −70°C until assayed.

The second half of the experiment was conducted at night. The animals entered darkness at the normal time (2000 hr). At 1.5 hr into the dark phase all animals were exposed to light (150–200 $\mu W/cm^2$) to suppress their rising pineal NAT activity and melatonin levels; light of this irradiance is sufficiently bright to totally suppress rat pineal NAT and melatonin within 20–30 min (12, 13). Twenty minutes after light onset (at 2150 hr), seven rats received a subcutaneous injection of DMI (10 mg/kg body wt) in 0.1 ml saline. At 2200 hr, these seven animals plus seven rats not treated with DMI began swimming as described above. Two hours after swimming onset the rats were removed from the water and their pineal glands were collected. At the same time, seven rats that were exposed to light but did not swim were killed and their pineal glands were also dissected. Pineals were frozen on solid CO_2 and stored as above.

Pineal NAT and HIOMT activities and radioimmunoassayable melatonin were determined in each gland using the method of Champney *et al.* (14). Briefly, pineal glands were disrupted by sonication in 100 μl of 0.5 *M* phosphate-buffered saline. Aliquots were then assayed, in duplicate, for the enzyme activity and melatonin content. Data were statistically analyzed using a one-way analysis of variance with significant differences between group means being determined using a Student–Newman–Kuels test. Data are expressed as means ± SE.

Results. When rats swam for 2 hr during the day (between 1400 and 1600 hr) pineal NAT activity rose significantly only in those animals that had been pretreated with DMI (Fig. 1). Conversely, HIOMT activity was not influenced by swimming whether or not the rats were treated with DMI. Daytime melatonin levels were significantly increased by swimming alone but the rise was much greater in the rats that had been treated with the NE uptake blocker, DMI.

When animals were forced to swim at night, NAT activity rose slightly after swimming alone but the rise was greatly exaggerated in the rats that had received DMI. As during the day, swimming at night did not alter HIOMT activity. Pineal melatonin levels responded somewhat differently to stress at night than during the day. Swimming even without DMI pretreatment caused a highly significant rise in pineal melatonin levels. Likewise, in the rats that were given DMI and then swam at night the melatonin rise was substantial but it was no greater than that caused by swimming alone.

There were clear differences between the pineal responses to a 2-hr swim during the day and night. In reference to pineal NAT, in

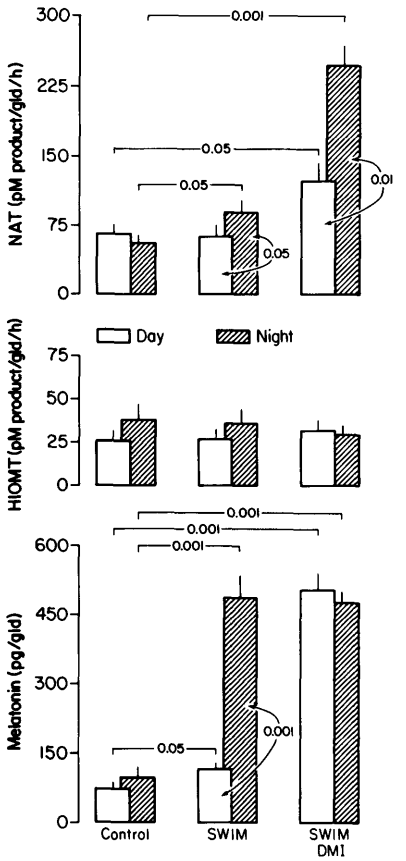

FIG. 1. Changes in rat pineal NAT, HIOMT, and melatonin after swimming for 2 hr either during the day or at night. The responses of both NAT and melatonin were much greater at night than during the day. Control, nonswimming controls; SWIM, swimming for 2 hr in 30°C water; DMI, treated with desmethylimipramine.

rats that swam only as well as those that were given DMI and swam the increases were significantly greater at night. Also, swimming at night without DMI pretreatment led to a large rise in pineal melatonin, a response not observed during the day. After DMI treatment, swimming caused equivalent rises in pineal melatonin during the day and at night.

Discussion. These findings confirm that aversive stimuli, such as swimming, during the day have a minor influence on pineal melatonin synthesis unless the animals are pretreated with a NE uptake inhibitor. Without the DMI pretreatment, excessive catecholamines that arrive at the pineal gland in the systemic circulation are sequestered in the sympathetic nerve endings and, as a consequence, the β-adrenergic receptors in the pinealocyte membrane are protected from stimulation and melatonin levels increase either minimally or not at all (11). When NE uptake is blocked, the catecholamine is accessible to the β-adrenergic receptors in the pinealocyte membrane and both NAT and melatonin levels rise significantly in response to daytime swimming (Fig. 1).

At night, however, a 2-hr swim by itself caused a modest rise in pineal NAT and a marked increase in melatonin. The greater increase in the pineal response to swimming stress at night possibly relates to the large increase in the number of β-adrenergic receptors in the pinealocyte membrane at night (3, 4). Using specific β-receptor ligands, Reiter *et al.* (3) and Gonzalez-Brito and colleagues (4) found that ligand binding at night increases by at least a factor of two compared to the day. Thus, NE arriving in the pineal at this time has access to an increased number of β-receptors and stimulates the cascade of events leading to melatonin production despite an active NE uptake mechanism by the sympathetic nerve endings.

It is conceivable that the differential response of the pineal gland to swimming during the day and night related not directly to the pineal gland but rather to the response of the adrenal medullae to stress at these two times; i.e., the catecholamine response to swimming may have been greater at night and, as a consequence, a larger increase in pineal melatonin resulted. Whereas the catecholamine response of rats to stress has been extensively studied during the daytime, the authors are not aware of any similar studies conducted at night. Thus, whereas it is possible that swimming led to larger increases in catecholamines at night and thereby to a greater melatonin response, the most likely explanation for the greater nighttime response relates to the increased number of β-receptors in the pinealocyte membrane during darkness (3, 4), as summarized above.

Another potential explanation for the greater stimulation of the pineal gland by swimming at night compared to the day in-

volves the neuronal uptake of NE. Although this has never been experimentally documented, it is possible that the catecholamine uptake mechanism is suppressed at night and, therefore, circulating catacholamines enter the synaptic cleft and act on the β-adrenergic receptors in the pinealocyte membrane resulting in the increased conversion of serotonin and melatonin. This possibility is supported by the nighttime pineal melatonin response to swimming. Pretreatment of the rats at night by DMI did not exaggerate the melatonin response to swimming suggesting that NE uptake was not a major factor at night. This was not, however, the case when NAT was measured. Thus, DMI-treated rats exhibited a greater pineal NAT response to swimming compared to those that only swam.

The lack of a change in HIOMT activity in response to swimming was not unexpected. HIOMT levels in the rat pineal seem to be very stable and levels remain virtually unchanged throughout a normal LD cycle (6). Apparently HIOMT activity in the rat pineal is always high enough to convert available substrate, in this case *N*-acetylserotonin, to melatonin (6).

The apparent dichotomous response of pineal NAT and melatonin to swimming (without DMI pretreatment) at night is not totally novel. At this time, swimming caused only a slight increase in pineal NAT while melatonin levels increased dramatically (Fig. 1). Although there is often a strong correlation between these two constituents, factors other than NAT may determine the quantity of melatonin formed (5, 15) and certainly there are examples in the literature for a lack of parallelism between pineal NAT activity and melatonin content (12).

In the present study the rats at night were exposed to 30 min of light before the imposition of the swimming stress. This was done to lower night melatonin levels to basal daytime values (13), so that the stimulus, in this case swimming, and the subsequent release of catecholamines could act on a pineal that was at its lowest level in terms of its NAT activity and melatonin content.

In summary, the rat pineal response to swimming stress is different during the day and at night. The results of earlier studies suggested that the pineal responded only weakly or not at all to peripherally circulating catecholamines (11). The present findings, however, indicate that this may be true during the normal light period but, conversely, stress at night leads to a substantial rise in pineal melatonin production. The findings suggest that the rise in the number of β-adrenergic receptors in the rat pinealocyte membrane during the daily dark period (3, 4) has physiological relevance in terms of melatonin production.

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