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THE SYRIAN HAMSTER PINEAL GLAND RESPONDS TO ISOPROTERENOL IN VIVO AT NIGHT

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ABSTRACT: Failure of isoproterenol (ISO) injections to raise pineal melatonin content has generated doubt about β-adrenergic control of the melatonin rhythm in Syrian hamsters. However, the effect of ISO injected at night after light-induced reduction of pineal melatonin has not been reported. In this study, light exposure began at 6 1/4 h into one (normally 10-h) dark phase. The hamsters were injected with either ISO (1 mg/kg) or vehicle 15 min later when pineal melatonin content was low. Light exposure continued. Two h after ISO but not vehicle injection, pineal melatonin content rose more than six-fold. In other animals injected at the end of the usual light phase then kept in light for 2 h, pineal melatonin was equally low after ISO or vehicle injection. The Syrian hamster pineal gland can respond in vivo to a β-adrenergic agonist injected at the physiologically relevant time of the normal nocturnal melatonin surge. This finding, taken together with the previously reported inhibition of the endogenous nocturnal melatonin surge with a β-blocking drug, suggests that a β-adrenergic mechanism controls the hamster pineal melatonin rhythm.

In rats, β-adrenergic agonists injected during daytime stimulate pineal melatonin synthesis (1-5). In contrast, Syrian hamster pineal melatonin does not rise in response to isoproterenol (ISO) in either the light (6-8) or dark (9) phase of the normal cycle. The purpose of this study was to determine if hamster pineal melatonin content rises after injection of ISO when the nocturnal melatonin surge is acutely blocked by light.

MATERIALS AND METHODS

Adult male Syrian hamsters (Mesocricetus auratus) were adapted for at least one month to a 14L:10D light cycle (lights off: 2000-0600 h). They were given a standard rodent chow and water ad libitum. Light ("cool white" fluorescent) intensity was 75 foot-candles at the front of the cages. Each experiment was done on a different night. The animals were killed by decapitation, and the pineal was removed, frozen on dry ice and sonicated in assay buffer. Homogenates were stored at -60 C until RIA of melatonin (10). Sampling in the dark was carried out under dim red light (two 25-watt incandescent globes, each shielded with a Kodak No. 1A filter). Results were analyzed by t tests with the Bonferroni correction for multiplicity of comparisons

In experiment (exp.) 1, 0.1 ml of either vehicle or ISO (1 mg/kg) was injected sc at the end of a light phase. Animals (8/group) were kept in light for 2 h until sampling.

In exp. 2, 5 hamsters were sampled in the dark at 0215 h. At this time, 31 other hamsters were transferred into a lighted room for subsequent sampling in order to determine when pineal melatonin content reached daytime levels. Animals were grouped for the time intervals after beginning the light exposure: 2 animals at 0-5 min, 3 at 5-10 min, 4 at 10-15 min, 3 at 15-20 min, 7 at 20-30 min, 9 at 30-40 min, and 3 at 40-50 min. A best-fit decay line was obtained by a single 3-parameter exponential regression of the individual data.

In exp. 3 (7-8/group), 2 groups of hamsters were sampled in the dark; one at 0215 h and the other 2 h later. Three other groups were brought into a lighted room at 0215 h; 1 was sampled 15 min later at 0230 h, and the other 2 were injected sc at 0230 h with 0.1 ml of either 0.15 M NaCl vehicle or ISO (1 mg/kg) and kept in light for 2 h until sampling. In exp. 4, groups of 7-8 hamsters were sampled with the same protocol as in exp. 3, except there was no group sampled at 0215 h in the dark.

RESULTS

ISO and vehicle injections at the end of the light phase resulted in similar pineal melatonin content (exp. 1, Fig. 1A), characteristic of normal daytime values in this laboratory (10, 11).

During the second half of the dark phase (Fig. 1B), by 15 min in novel light exposure, pineal melatonin content in uninjected animals (exp. 2) was typical for the end of the light phase (11). Hamsters sampled 2 h after receiving saline had pineal melatonin contents not different from values expected at the time of injection. 2 hours after injection of ISO, the values were significantly higher ($P < 0.001$) than those obtained either after saline or at the time of injection (combined exp. 3 and 4). The values for hamsters killed in the dark were typical of those found during the normal nocturnal surge of pineal melatonin synthesis (10, 11).

DISCUSSION

In a previous study of Syrian hamsters (7), injection of 3 or 10 mg/kg ISO sc during the day (4-9 h into the light phase) produced no response in pineal melatonin content assessed at intervals from 40 min to

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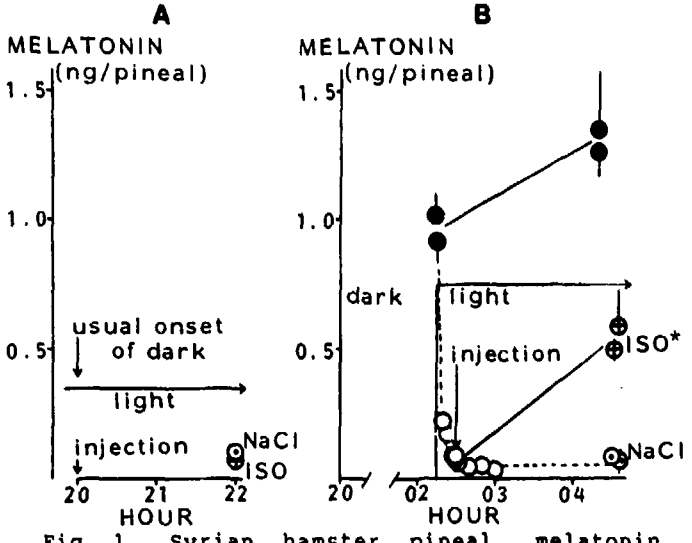

Fig. 1. Syrian hamster pineal melatonin content (mean + SE): A (exp. 1), after isoproterenol (ISO, 1 mg/kg) or saline vehicle (NaCl) injected at the end of the usual light phase; B (exp. 2-4), after injection of ISO (crossed circles) or NaCl (dotted circles) or no injection (open circles) during exposure to light from 0215 h, or after no injection and no nocturnal light exposure (closed circles). The dashed line represents the best-fit curve for the data of exp. 2 with a decay constant of -0.31/min and half-time of 2 1/4 min. *$P < 0.001$, combined ISO groups vs. those having received NaCl or those sampled at 0230 h without injection.

5 h after injection. When 3 or 6 mg/kg of ISO was injected at the end of the light phase or in the dark phase, there was no detectable advancement or enhancement of the nocturnal melatonin surge (9). Such results seem incongruent with the previously shown ability of pharmacologic β-blockade to prevent the normal in vivo nocturnal surge of pineal melatonin content in this species (12). However, norepinephrine (NE) released from the intrinsic nerve endings probably is highly concentrated at the pinealocyte during the nocturnal surge of pineal sympathetic activity (11, 13) and may allow little additional influence of injected ISO. We were interested in whether ISO, injected at night, could raise pineal melatonin from light-induced low levels before insensitivity might develop. Previous in vitro studies (14) had indicated that light exposure of hamsters for 6 h at night inhibited a response of pineal melatonin synthesis during incubation with NE.

After blocking neuronal uptake with desipramine, we previously stimulated a rise in hamster pineal melatonin by injecting NE 30 min following onset of lighting at night (14; Reiter, Vaughan, Oaknin, unpublished results). In the present study, we used ISO because it is not inactivated by neuronal uptake and does not require use of another drug for blockade of uptake in pineal nerve endings (15), and the α activity of NE might influence pineal melatonin synthesis (16). Thus, we injected ISO 2 h before the normal time of the peak melatonin content and after the shortest light exposure able to depress melatonin to daytime levels. Injected at 0230 h after 15 min light, ISO produced a 6.7-fold rise in pineal melatonin content. Since no effect was seen after ISO given at 2000 h, it appears that sensitivity to ISO develops some time during the dark phase. The insensitivity of the hamster pineal at the end of the light phase appears impressive. Pineals taken then and incubated with doses of ISO up to 10 μM had no response of melatonin synthesis, yet those taken after brief light exposure 6 h into the dark phase had a dramatic response beginning with ISO concentration at least two logs lower (17). The present results show that a difference between these two time points can be demonstrated in vivo.

In a previous report, ISO (10 mg/kg ip) injected in the dark, when pineal melatonin was high, inhibited a fall in hamster pineal melatonin content after subsequent light exposure (9). Though this is in agreement with the present finding of nocturnal responsiveness to ISO, those studies did not include injection of ISO at night shortly after light exposure, and thus did not demonstrate the ability of ISO to stimulate pineal melatonin from low levels. The present results demonstrate this and help substantiate the view that in Syrian hamsters, as in rats, the pineal melatonin rhythm is controlled by a β-adrenergic mechanism.

These findings differ markedly from the reported peak responsiveness of the pineal at the end of the light phase when there has been the least endogenous stimulation of the pineal and the least down-regulation of catecholamine receptors on pinealocytes (3-5, 18). These reports of rats with denervated pineals or of intact rats during a normal or prolonged light phase did not assess responsiveness during the time of the nocturnal melatonin surge after exposure to darkness for part of the night. In fact, in vitro results indicate that even in the rat, which maintains easily demonstrable pineal β-responsiveness during the day, pineal glands taken 6 1/2 h into the night after a period of darkness are more responsive than those taken at the end of the day (17). Our results in the hamster substantiate this view. Whether the nocturnal responsiveness in hamster pineal glands results from a change at the receptor or post-receptor level is unknown, though catecholamine receptor density apparently increases at night in rat pineal (19).

The elevated pineal melatonin content after nocturnal ISO injection did not reach the level seen during the endogenous nocturnal surge. This might be due in part to a sub-optimal dose of ISO and/or time of injection or sampling. Dose-response trials for different times of ISO injection or of sampling have not been performed, though experiments with NE injection after brief light exposure in desipramine-primed hamsters indicate that the period of maximal sensitivity may be the second half of the dark phase (Reiter, Vaughan and Oaknin,

unpublished results). Another factor, already suggested (17), may be that "the brief light exposure itself before and during the action of the injected agonist might have partially induced insensitivity of the pineal. Besides the nocturnal stimulatory effect of ISO on hamster pineal melatonin synthesis (17), ISO may also minimally enhance elimination of melatonin from the pineal at night as suggested indirectly by in vitro results (17). Whether such an effect might have dampened the rise in melatonin content after in vivo ISO injection at night is not known.

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