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Lesions of the Melatonin- and Androgen-Responsive Tissue of the Dorsomedial Nucleus of the Hypothalamus Block the Gonadal Response of Male Syrian Hamsters to Programmed Infusions of Melatonin[1]

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ABSTRACT

The objective of this study was to characterize a site at which it is likely that melatonin mediates photoperiodic control of reproduction in the male Syrian hamster. The first experiment was a comparison of the distributions of iodomelatonin (IMEL)-binding sites and cells immunoreactive to androgen receptors (AR-ir) in the medio-basal hypothalamus (MBH). AR-ir cells extended throughout the MBH, whereas IMEL binding was restricted to the dorsomedial nucleus (DMN). Comparisons between IMEL binding and AR-ir on adjacent cryostat sections revealed a clear overlap between the IMEL-binding sites and a distinct subpopulation of AR-ir cells within the DMN. The second experiment examined whether lesions of these IMEL- and androgen-responsive cells affected the response of the hamsters to short-day (SD)-like infusions of melatonin. Animals received sham or bilateral electrolytic lesions of the IMEL-binding sites within the DMN of the hypothalamus (MBH-X). Animals were pinealectomized and 4 wk later fitted with an s.c. cannula for the daily infusion of either melatonin (50 ng/h) or saline (500 μl/10 h). After 6 wk the animals with sham lesions showed gonadal atrophy and lower serum concentrations of LH and prolactin (PRL) after infusions with melatonin. In contrast, MBH-X animals given melatonin had large testes and long-day (LD)-like serum LH concentrations. Infusions of melatonin did, however, cause a significant decline in serum PRL levels. This study shows that an intact MBH is essential for the expression of gonadotrophic but not lactotrophic responses to melatonin and/or photoperiod. It also suggests that cells responsive to both gonadal steroids and melatonin may be involved in the seasonal variation in GnRH release, and indicates a site at which melatonin might influence sensitivity to steroid feedback, a hypothalamic function known to be regulated by photoperiod.

INTRODUCTION

The mammalian pineal gland mediates seasonal adaptations to the environment, including reproduction, thermogenesis, moult, torpor, and associated behaviors [1–4]. The secretion of melatonin from the pineal is under direct control of the circadian system such that the duration of the nocturnal rise in serum melatonin is directly proportional to the length of the night [3–5]. The influence of day length on the reproductive axis can be blocked by pinealectomy and replicated by appropriate administration of melatonin, the duration of the signal being its critical parameter [6, 7]. However, the sites and mechanisms of action of melatonin in mediating these seasonal changes in reproduction have not been identified. Since changes in both the feedback effects of steroids and GnRH secretion, and hence LH secretion, occur throughout the season in the absence of marked changes in responsiveness of the pituitary, photoperiodic influences must involve a central site of action [8]. In support of this hypothesis, central administration of melatonin into the medial hypothalamus of a variety of rodents prevents photoperiod-induced changes in the hypothalamo-pituitary-gonadal axis [9–12]. Additionally, microimplants of melatonin into the medio-basal hypothalamus (MBH), but not other central sites, of the sheep block the inhibitory effects of long days on reproduction [13–15]. Autoradiographic studies, using 2-[^{125}I]iodomelatonin (IMEL) as the radioligand, have revealed specific, high-affinity binding sites within the brain and pituitary of photoperiodic species [16–18]. In the Syrian hamster, IMEL binding has been identified in the preoptic area (POA), suprachiasmatic nucleus (SCN), MBH, paraventricular nucleus of the thalamus, and the pars tuberalis of the pituitary (PT). Studies involving lesions directed toward these neural areas independently have revealed that only those lesions that ablated the IMEL-binding sites within the MBH blocked gonadotrophic responses to short photoperiods and exogenous melatonin [6, 19–21]. Since the distribution of IMEL binding within the MBH of the Syrian hamster has been described as located within either the dorsomedial division of the ventromedial nucleus (VMN) or the dorsomedial nucleus (DMN), one of the objectives of the present study was to determine which nuclei within the MBH contain these melatonin-responsive cells [17, 18].

The phenotype of melatonin-responsive cells is unknown. Melatonin could regulate GnRH neurons directly or could modulate negative feedback effects of testosterone through an influence on androgen-responsive neurons [22, 23]. The former possibility seems unlikely in view of the mismatch between the localization of IMEL-binding sites and the distribution of cells immunoreactive for GnRH [24]. The hypothesis of an indirect action is favored not only be-

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cause of the presence of both androgen receptors and IMEL-binding sites within the MBH, but also because of evidence that GnRH cells generally lack receptors for steroid hormones [25, 26]. To ascertain whether or not melatonin target cells essential to seasonal reproduction may also respond directly to steroids, we investigated the localization of androgen receptor immunoreactivity (AR-ir) and compared it to the distribution of IMEL-binding sites on alternate sections through the MBH.

The second objective of this study was to confirm and extend a previous observation that lesions of the IMEL-binding sites within the MBH blocked gonadotrophic, but not lactotrophic, responses to short photoperiods (SD) and exogenous SD-like infusions of melatonin [19]. In the earlier study the animals were exposed to SD before being infused with melatonin, so there was a possibility that they were scotorefractory at the time they received melatonin. Although considered unlikely, this could explain the absence of a gonadotrophic response to exogenous melatonin. In the present experiment, to control for any effects of SD on scotorefractoriness, animals were maintained on long days (LD) for the entire experimental period while receiving an SD-like s.c. infusion of melatonin. If the tissue bearing those binding sites is a necessary component to the pathway mediating seasonal gonadal responses to melatonin, then lesions of the IMEL-binding sites within the MBH will block the effects of the melatonin infusion.

MATERIALS AND METHODS

General

Adult male Syrian hamsters (Wrights of Essex, Chelmsford, UK), 100–120 g BW, were housed 8 per cage with food and water available ad libitum under an LD photoschedule of 16L:8D (lights-off at 2000 h) for at least 4 wk before the study. Procedures were licensed under the Animals (Scientific Procedures) Act 1986 and were conducted in accordance with the University of Cambridge Scientific Procedures on Animals Code of Practice.

Experiment 1: Comparison between the Distribution of IMEL Binding and AR-ir

Animals were killed by cervical dislocation (n = 3), and the brains were removed and rapidly frozen on dry ice. The brains were stored at −20°C before being cut into 16-μm sections with a cryostat. Alternate series of adjacent sections through the MBH were collected onto separate gelatinized double-subbed slides [27]. Sections for immunocytochemistry (ICC) were thaw-mounted directly onto the slide, which was kept on a hot plate at 37°C until all sections were dry (up to 5 min). Each slide was then immediately fixed in 4% paraformaldehyde/0.01 M PBS for 1 h, washed three times (5 min each) in 0.01 M PBS, and then processed for ICC. The primary antiserum for the androgen receptor (PG21–18), kindly donated by Dr. Gail Prins, was used at a final dilution of 1:500. Its validation has been described elsewhere [28, 29]. The PG21–18 antiserum appears to bind specifically to the androgen receptor after binding of either testosterone or 5α-dihydrotestosterone [29]. Immunoreactivity was visualized using an indirect biotin-avidin protocol (Vectastain Elite, Peterborough, UK) with diamino-benzidine (DAB; Sigma, Poole, UK) as the chromogen.

The alternate sections collected for in vitro autoradiography of IMEL-binding sites were thaw-mounted directly onto slides at room temperature and then held at 4°C until an entire brain had been sectioned. They were then stored at −50°C with desiccant for up to 4 wk. Autoradiography was carried out according to the method described by Williams et al. [18]. Briefly, thaw-mounted sections were incubated in Tris-HCl buffer (pH 7.4) at room temperature for 10 min and then for 60 min in Tris buffer containing 100 pM [^{125}I]iodomelatonin (IMEL, 2000 Ci/mmol; Amersham, Aylesbury, UK). Slides were washed three times in ice-cold Tris, dipped in distilled water, air dried, and then apposed to Hyperfilm (Amersham) for 20 days. Slides generating the image were stained with cresyl violet, and the anatomical location of the melatonin binding was assessed relative to landmarks identified by the staining.

To assess the overlap between the distribution of IMEL after in vitro film autoradiography and localization of AR-ir using ICC, camera lucida drawings of the cresyl violet sections used to generate the IMEL images were compared with the distribution of AR-ir in the alternate sections. From the size and shape of the brain, there appeared to be no differences between the procedures in the extent of any shrinkage of the tissue; thus it was possible to overlay directly the images drawn from the position of both the IMEL binding and the AR-ir.

Experiment 2: Effect of SD-Like Infusions of Melatonin in LD-Housed Animals Bearing Lesions of the MBH

All surgery was carried out under Avertin anesthesia (tribromo-ethanol/tertiary amyl alcohol; 1 ml/100 g BW, i.p.). Bilateral electrolytic lesions (2 mA, 10 sec) were made with stainless steel electrodes directed at the IMEL-binding sites within the MBH (MBH-X) [19]. Coordinates were as follows: 0.17 cm anterior to bregma, ± 0.06 cm lateral, 0.7 cm deep to dural surface, incisor bar + 0.5 cm above ear bar. Controls received sham lesions (no current passing through the electrodes). After 4 wk of recovery on LD, animals were pinealectomized as described by Hoffman and Reiter [30]. One week after pinealectomy, hamsters were fitted with an s.c. cannula attached via a fluid swivel to an infusion pump (Harvard Instruments, Edenbridge, UK) controlled by an electronic timer. Animals received 10-h infusions of either

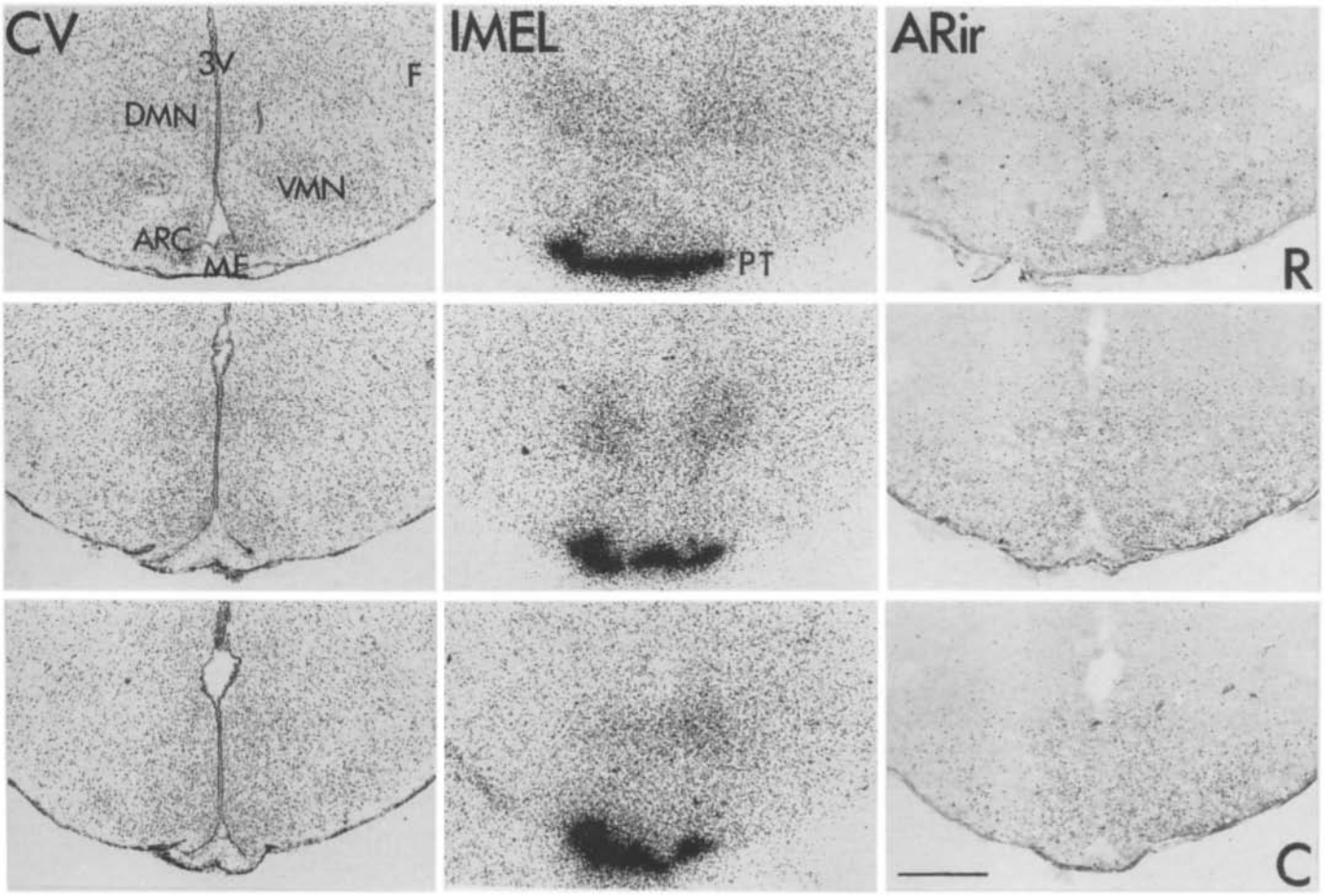


FIG. 1. Photomicrographs showing the rostro-caudal distribution, within coronal sections (16 μm) of the MBH of the Syrian hamster, of cresyl violet (CV)-stained sections used to generate the IMEL images and AR-ir. Abbreviations: C = caudal; R = rostral; ME = median eminence; F = fornix; 3V = third ventricle; DMN = dorsomedial nucleus; VMN = ventromedial nucleus; ARC = arcuate nucleus; PT = pars tuberalis. Bar = 1 mm.

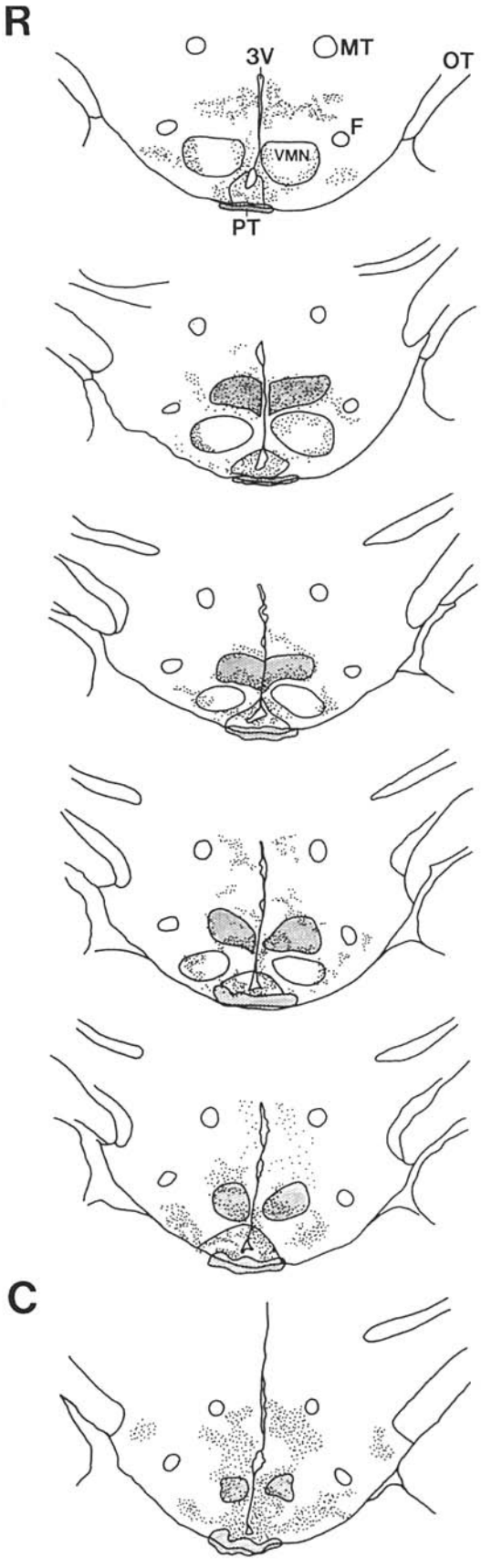
saline vehicle or melatonin (1 mg/ml; Sigma, Poole, UK) dissolved in 0.1% ethanolic saline at a rate of 50 μl/h, i.e., 500 ng/night for 6 wk, a rate shown previously to restore physiological levels of circulating melatonin [31].

For verification of the placement of lesions, at the end of the 6 wk of infusions all MBH-X animals were killed by anesthesia with Euthatal (Rhone Merieux, Essex, UK; 0.5 ml/100 g BW, i.p.), and were perfused transcardially with 4% paraformaldehyde in 0.01 M PBS. Their brains were removed, postfixed for 2 h, and then transferred to a 20% sucrose-0.01 M PBS solution overnight. Brains were then cut on a freezing microtome at 40 μm and stained with cresyl violet. Only those animals bearing complete bilateral ablation (n = 10) of these sites were included in the analyses.

Blood was collected via cardiac puncture under light ether anesthesia and left to clot overnight at 4°C. After centrifugation, serum samples were stored at −20°C before RIA for the measurement of serum LH and prolactin (PRL). LH concentrations were determined using an NIDDK (Baltimore, MD) kit for rat LH, with RP-3 as the reference preparation. Samples were assayed in duplicate in the same assay; the limit of detection was 0.1 ng/ml (with 100 μl serum) with an intraassay coefficient of variation of 6.8%. PRL concentrations were determined in a homologous assay using an antiserum against hamster PRL provided by Dr. F. Talamantes [32]. The purified hamster PRL used for iodinated tracer and standard preparation AFP-10302E was obtained from the NIDDK kit supplied by Dr. A.F. Parlow (Harbor-University of California, Los Angeles, CA). Samples were assayed in duplicate within the same assay; the limit of detection was 0.78 ng/ml (with 100 μl serum), and the intraassay coefficient of variation was 5.9%. Undetectable levels were assigned the limit of detection for each assay.

Statistics

All analyses were performed with the Statview II application (Abacus Concepts, Berkeley, CA) run on an Apple Macintosh personal computer (Apple Computer Inc., Cupertino, CA). Differences between treatment groups were assessed by two-way ANOVA and post hoc Dunnett's *t*-test.



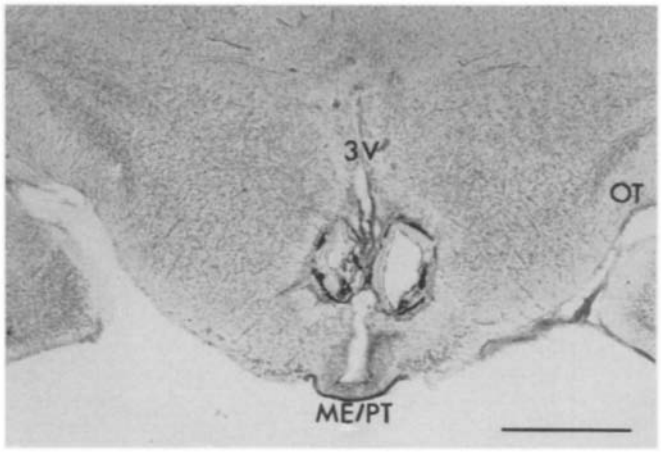


FIG. 3. Photomicrograph showing a representative electrolytic lesion directed toward the IMEL- and AR-responsive cells within the MBH. Abbreviations: ME = median eminence; PT = pars tuberalis; 3V = third ventricle; OT = optic tract. Bar = 1 mm.

RESULTS

Experiment 1: Comparison between Distribution of IMEL-Binding Sites and AR-ir Cells in the MBH

As anticipated, IMEL binding was identified at a number of hypothalamic sites: POA, SCN (data not shown), and MBH. Within the MBH, the IMEL binding was restricted to the compact region of the DMN and was present throughout the rostro-caudal extent of the nucleus (Fig. 1). No binding was evident in the VMN or the median eminence, but there was very intense binding of IMEL to fragments of PT tissue adherent to the base of the brain.

Immunoreactivity for the AR was restricted to the cell nuclei; no cytoplasmic staining was observed. The intensity of the immunostaining appeared heterogeneous, ranging within the same section from very intense to light (Fig. 1). AR-ir cells were identified throughout the hypothalamus extending caudally from the POA and into the lateral hypothalamic area. There were also groups of immunopositive cells within the ventrolateral and dorsomedial divisions of the VMN, medial tuberal nucleus, arcuate nucleus, and median eminence/PT, and there were scattered immunopositive cells throughout the rostro-caudal extent of both the compact and diffuse regions of the DMN.

By comparing the distributions of IMEL binding with AR-ir on adjacent tissue sections, it was possible to map any areas of overlap between the two markers (Fig. 2). There was overlap between the IMEL-binding sites and the AR-ir within the POA (data not shown). However, within the

FIG. 2. Camera lucida drawings showing the area of overlap between the IMEL-binding sites (shading) and AR-ir (dots) in the medio-basal hypothalamus. Abbreviations: C = caudal; R = rostral; ME = median eminence; F = fornix; 3V = third ventricle; OT = optic tract; PT = pars tuberalis; MT = mammilothalamic tract.

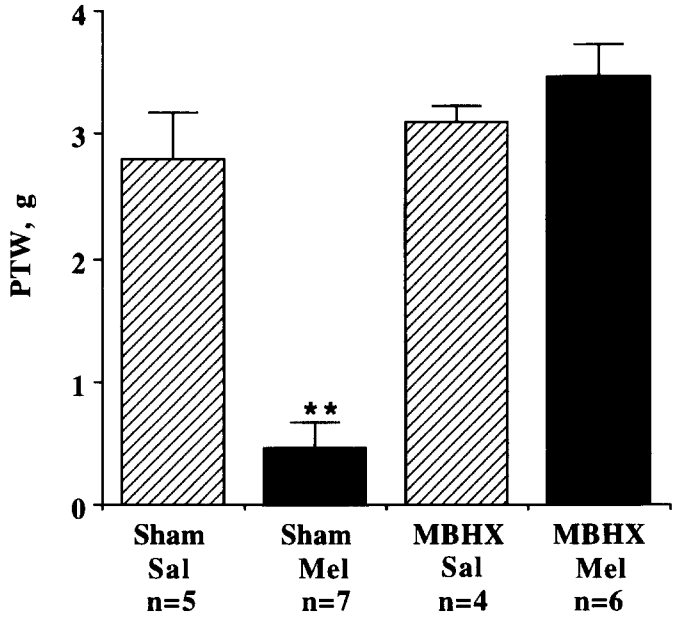


FIG. 4. Paired testicular weights (PTW; mean ± SEM) of sham lesion-bearing or MBH-lesion-bearing male Syrian hamsters housed under an LD photoschedule (16L:8D) and given programmed infusions of saline vehicle (50 μg/h) or an SD-like melatonin signal (10 h; 50 ng/h) once daily for 6 wk. $^{**}p < 0.01$ vs. sham lesion-bearing saline group.

MBH there was a distinct pattern of heavily stained AR-ir cells in the ventral division of the compact region of the DMN, corresponding directly to the area containing IMEL-binding sites. No other sites with comparable dual localization were found within the MBH; therefore, the subsequent lesion study focused on this area.

Experiment 2: Gonadal Responses of LD-Housed Animals Bearing MBH Lesions to SD-Like Infusions of Melatonin

Only animals bearing complete bilateral ablation of the IMEL-binding sites within the MBH, as verified by histological examination postmortem, were included in the analysis. The lesions ablated the dorsomedial division of the VMN and the ventromedial area of the DMN in addition to the intervening tissue, with no evident damage to the median eminence or arcuate nucleus (Fig. 3). There was no significant difference between the final body weights of the animals with sham lesions and the MBH-X animals, irrespective of whether they had received saline or melatonin infusions. However, after infusion of melatonin or saline vehicle once daily for 6 wk to animals with sham lesions and MBH-X animals (Fig. 4), there were highly significant effects on the paired testes weights of the lesion ($F = 44.38$; df 1,18; $p < 0.01$) and the infusion ($F = 11.44$; df 1,18; $p < 0.01$) as well as a highly significant interaction ($F = 22.65$; df 1,18; $p < 0.01$) between the two variables. Animals bearing sham lesions showed the expected gonadal regression in response to the infusion of melatonin, whereas the testes remained large in sham lesion-

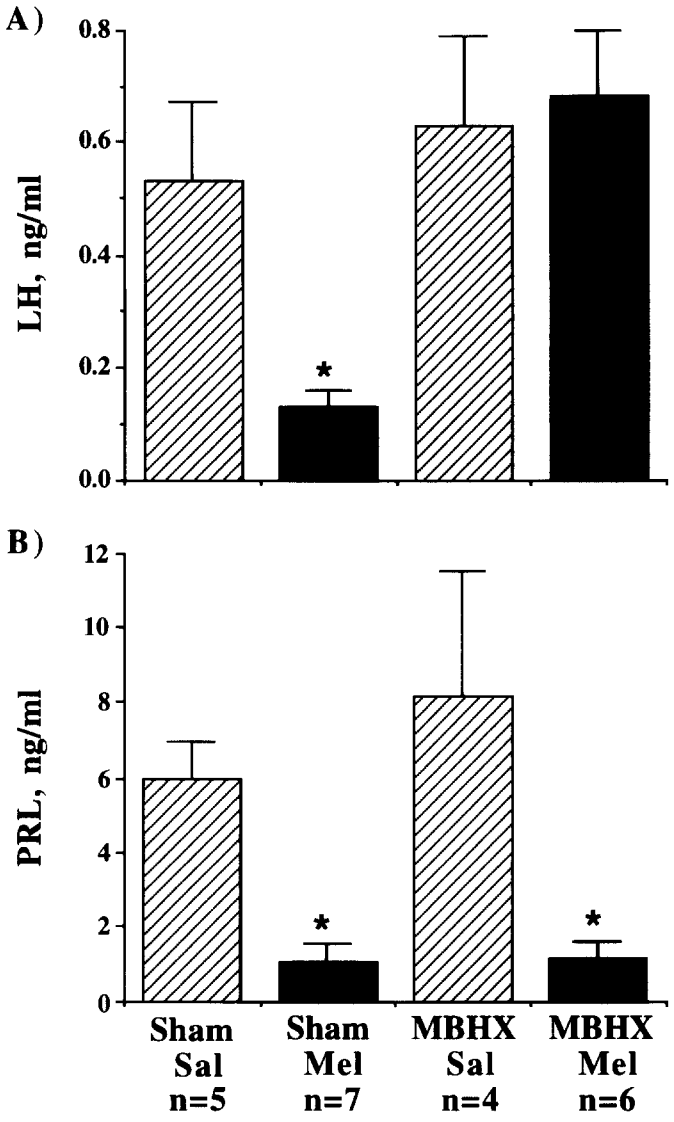


FIG. 5. The effect of 6 wk of daily 10-h s.c. infusions of either melatonin or saline into pinealectomized sham lesion-bearing or MBH-lesion-bearing male Syrian hamsters on (A) serum LH and (B) PRL concentrations (mean ± SEM). * $p < 0.01$ vs. sham saline group.

bearing animals that had received saline infusions. Similarly, infusion of saline to the MBH-X animals had no effect, and the testes remained large. However, infusion of melatonin did not induce testicular regression in any animal bearing lesions; the testes of these animals were significantly larger than those of the sham lesion-bearing animals that had received melatonin ($p < 0.01$).

Measurements of serum LH (Fig. 5a) showed a highly significant effect of lesion ($F = 8.39$; df 1,18; $p < 0.01$) but not infusion ($F = 2.89$; df 1,18; $p > 0.1$), but there was also a significant interaction ($F = 4.9$; df 1,18; $p < 0.05$) between these variables. Animals with sham lesions showed significantly lower serum LH concentrations after infusions of

melatonin compared to saline ($p < 0.01$). However, there was no significant difference in serum LH concentrations between sham lesion-bearing animals that had received saline and MBH-X animals. Furthermore, both saline- and melatonin-infused groups of MBH-X animals had LD-like LH concentrations that were not significantly different from each other or from those for the sham lesion-bearing saline-infused group. The saline- and melatonin-infused MBH-X animals had significantly higher serum LH levels than the melatonin-infused animals with sham lesions ($p < 0.01$).

Analysis of serum PRL (Fig. 5b) concentrations revealed a significant effect of the infusion ($F = 19.96$; df 1,18; $p < 0.01$), but not the lesion ($F = 0.78$; df 1,18; $p > 0.3$); and there was no interaction ($F = 0.6$; df 1,18; $p > 0.4$) between the two. In contrast to the effects on LH, animals receiving an SD-like infusion of melatonin had significantly lower circulating PRL levels both in the group with the sham lesions and in the MBH-X group compared to their saline-infused controls ($p < 0.01$). The lesion therefore dissociated melatonin-dependent control of the gonadotrophic and lactotrophic axes. In addition, these data demonstrate that the IMEL-binding sites within the DMN of the MBH are essential for the effects of melatonin in the photoperiodic control of gonadotrophic, but not lactotrophic, function.

DISCUSSION

This study provides further evidence that in the Syrian hamster, melatonin mediates seasonal responses to photoperiod within the MBH, specifically via the IMEL-binding sites located within the DMN. In addition, a detailed description of AR-ir distribution, and comparison with the distribution of IMEL binding in the MBH of the adult male Syrian hamster, reveals distinct regions of overlap. Together these results suggest that an area responsive to both gonadal steroids and melatonin may regulate seasonal reproduction and that melatonin may influence sensitivity to steroidal feedback by acting directly on these cells.

Gonadal steroids have a profound role in seasonal influences on the reproductive hormones of the hypothalamo-pituitary axis. However, dual-staining techniques have shown that steroidal binding sites are not found in neurons that contain GnRH [25, 26], and studies in the Syrian hamster suggest a mismatch between the distribution of GnRH immunoreactivity and IMEL-binding sites [24]. This implies that effects of melatonin and steroid on GnRH secretion must be indirect, i.e., via other neural systems that are themselves targets for melatonin and/or steroid hormones and that converge onto GnRH neuronal cell bodies or terminals. Therefore, the strategy of the current study was to evaluate whether melatonin-responsive tissue also expressed AR-ir. Immunoreactivity for AR was restricted to the nuclear compartment, with no cytoplasmic staining as has been reported in some studies (e.g., Lehman et al. [33]). This may reflect the relative sensitivity of the method employed here, as the tissues were not fixed by perfusion prior to death. Nevertheless, the distribution of AR-ir neurons in the MBH of the male Syrian hamster reported in the present study is similar to that described for androgen/estrogen receptor-ir cells from other species [33–35]. Close examination of the distributions of AR-ir and IMEL-binding sites on adjacent sections did reveal significant areas of overlap within the DMN. To date little is known of the functional significance or cellular identity of cells within this nucleus. Until more sensitive techniques enabling the cellular localization of melatonin-responsive cells (such as in situ hybridization for the melatonin receptor [36]) can be combined with ICC for the AR, it will not be possible to know whether these two hormones act directly on the same cells or whether in this way the seasonal melatonin stimulus is able to control the sensitivity to steroids. Nevertheless, the DMN represents a point of convergence to melatonin (photoperiod) information and gonadal feedback. It may be that appropriate melatonin signals can alter the level and/or sensitivity of AR within the DMN. To date various groups have failed to demonstrate a consistent photoperiod-dependent change in steroid receptor concentration that could account for the effects of day length on behavioral or feedback influences of gonadal steroid hormones [37–39]. It may therefore be important to concentrate receptor/binding studies more directly on the DMN as a potential site for the photoperiodic mediation of steroidal feedback in this species.

A marked seasonal change in the negative feedback effect of testosterone has been demonstrated in the hamster, with the steroid being maximally effective during SD [22]. The anatomical data shown here, coupled with the inconsistent reports of effects of steroids on GnRH levels, suggest that gonadal steroids may be involved in the regulation of GnRH release rather than its synthesis [E. Bittman, unpublished results]. Thus steroids could regulate the expression of genes whose products regulate GnRH release, directly or indirectly. Candidates for such a mechanism could include dopamine, γ-aminobutyric acid, glutamate, neuropeptide Y, and the opioid β-endorphin. Gonadal steroid feedback could therefore affect the synthesis, turnover, and release of, for example, opioid-containing/steroid-concentrating fibers that ultimately project to the POA/septal region, which then influences the secretion of GnRH and therefore LH release from the pituitary [40, 41]. It has been shown that SDs are associated with an increase in β-endorphin concentration in the MBH of the Syrian hamster [41, 42]. However, it is not clear whether the neural or hormonal signals that influence the coordinated release of GnRH, and hence its effects on the reproductive axis, are at the level of the cell bodies or median eminence or both. Studies in rodents have shown connections between the DMN and lateral septal nucleus, POA, and arcuate/median eminence region [43], areas that also have high concentrations of steroid receptors; this

supports the idea of steroid-sensitive networks within the brain. There are also extensive reciprocal connections between the hypothalamic areas that bind IMEL (SCN, POA, DMN). It may be, therefore, that these hormones can act to modulate seasonal changes in reproduction and behavior. If the DMN participates in seasonal changes in brain sensitivity to steroid negative feedback, lesions of the IMEL-binding (and AR-ir) sites within the DMN may therefore hinder the induction of increased sensitivity to steroid negative feedback necessary for termination of the breeding season. It is therefore essential that future work concentrate on identifying the mechanism(s) that underlies the interaction between gonadal steroids and melatonin. This will involve a complex approach of combining ICC and in situ hybridization to identify the phenotype of the IMEL- and AR-sensitive cells and determining their connectivity.

In a previous study it was shown that hamsters bearing bilateral lesions to the IMEL-binding sites within the MBH did not undergo testicular regression in response to either inhibitory photoperiods or SD-like infusions of melatonin [19]. One interpretation is that cells or fibers in the area of the DMN play a role in tonic suppression of gonadotropin secretion and so the effects of these lesions on photoperiodic responses might be equivalent to those of olfactory bulbectomy or thyroidectomy [44, 45]. However, bulbectomized hamsters ultimately respond to melatonin injections [44], whereas the MBH-X animals in this and the previous experiment did not respond to melatonin infusions [19]. Although the animals in the earlier study had been exposed to SD for 12 wk before being infused with an SD-like melatonin signal [19], the reason they failed to respond to these infusions was not that they were already scotorefractory, since the MBH-X animals in the current experiment were maintained on an LD photoschedule throughout. In agreement with the previous study, these animals did not exhibit gonadal regression in response to SD-like infusions of melatonin, nor did infusion of melatonin result in lower serum LH concentrations compared to those in sham lesion-bearing animals infused with melatonin.

General photoperiodic competence of the MBH-X animals was not compromised, because as in the previous experiment [19], serum PRL levels were suppressed by infusions of melatonin. This dissociation between the melatonin-induced control of the secretion of PRL and LH underlines the fact that melatonin appears to influence PRL via a different site of action, independent of the DMN [46–48]. This may be via the PT of the pituitary. Hypothalamic-pituitary-disconnected rams, in which central regulation of the pituitary is abolished, are nevertheless able to show cycles of PRL secretion in response to changes in photoperiod when they are maintained under alternating 16-wk periods of LD and SD [49]. Such a mechanism would survive lesions of the DMN and might mediate the effects on PRL in the present study. In conjunction with data from pituitary disconnection studies and investigations using melatonin implants directed toward the MBH of rodents and sheep [9–15], as well as the presence of high-affinity IMEL-binding sites in the MBH region [17, 18], there is now compelling evidence to support the hypothesis that melatonin acts via IMEL-binding sites within the DMN to control seasonal reproduction in the Syrian hamster. However, the precise mechanism(s) by which melatonin achieves this has yet to be elucidated. Nevertheless, binding within the DMN is not consistent across species, and it may be that melatonin can act at other neural IMEL-binding sites to mediate seasonal control of gonadal status [10, 50].

In summary, these data extend and confirm the observation that the IMEL-binding sites within the DMN are essential for the mediation of photoperiodic time measurement in the Syrian hamster. Furthermore, the overlap of IMEL-binding sites with a distinct subpopulation of AR-ir cells within the DMN suggests that this could be a site at which melatonin might influence sensitivity to steroidal feedback.

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