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The neural basis of seasonal reproduction

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Summary — Many mammals have evolved seasonal patterns of reproduction and physiology which ensure adaptation to predictable annual changes in the environment. Domestication has led to the loss or diminution of seasonal responses in cows and pigs, but marked seasonal reproductive patterns are still evident in sheep, goats, horses and deer. The annual change in day length provides the major cue timing reproductive activity. The pattern of melatonin secretion from the pineal gland is the endocrine transducer of photoperiod, and thus regulates hypothalamic and pituitary mechanisms which control seasonal patterns of reproduction, pelage, appetite and growth. A pronounced daily rhythm of melatonin secretion occurs; high levels occur at night but little or no secretion occurs during the day. The rhythm of melatonin secretion persists with a period of ≈ 24 h in animals that are kept in constant darkness, *ie* it is an endogenous circadian rhythm. The rhythm of neural activity which drives melatonin secretion from the pineal is generated in the suprachiasmatic nucleus of the hypothalamus (SCN), and neural inputs reach the pineal gland *via* the sympathetic nervous system. Light, perceived by the retina, synchronises the endogenous rhythm to the external day–night cycle, and sets the duration of nocturnal secretion such that it is directly proportional to the length of the night. The change in duration of nocturnal melatonin secretion is critical in conveying seasonal information. The site and mechanism of action of melatonin within the brain remains largely a matter of conjecture. The secretion of luteinizing hormone and follicle stimulating hormone from the pituitary gland which controls gonadal function is regulated by secretion of gonadotrophin releasing hormone (GnRH) from the median eminence of the hypothalamus. The cell bodies of the GnRH neurons which project to the median eminence are located in the preoptic area and basal forebrain. Autoradiography using radiolabelled melatonin reveals that its receptors are mainly distributed in separate areas from those containing GnRH neurons. Thus, the actions of melatonin on GnRH secretion are probably mediated by interneurons. The neurochemical identities of such pathways are unknown, though catecholamine and endogenous opioid systems have been identified as inhibitory regulators of GnRH secretion, and there is currently considerable interest in glutamatergic neurons as stimulatory inputs for GnRH secretion. The actions of melatonin provide a challenge for the neuroscientist, particularly because its actions are entirely dependent on its pattern of secretion rather than simply its presence or absence. Study of melatonin also has pragmatic value, because its manipulation provides a means of regulating seasonality under field conditions.

melatonin / reproduction / GnRH / LH / FSH

Résumé — Bases neuro-endocriniennes de la reproduction saisonnière. *Beaucoup de mammifères ont développé un comportement saisonnier de reproduction et une physiologie qui permettent l'adaptation aux changements annuels prévisibles de leur environnement. La domestication a*

conduit à la perte ou la diminution du saisonnement chez les bovins et les porcins, mais un saisonnement marqué est toujours évident chez les ovins, caprins, équins et cervidés. Le changement annuel de la durée du jour fournit le stimulus principal qui synchronise l'activité de reproduction. Le profil de sécrétion de mélatonine par la glande pinéale est le traducteur endocrinien de la photopériode qui régule les mécanismes hypothalamiques et hypohysaires contrôlant l'évolution saisonnière de la reproduction, du pelage, de l'appétit et de la croissance. On constate un rythme quotidien prononcé de la sécrétion de mélatonine, avec des niveaux élevés pendant la nuit et peu ou pas de sécrétion pendant la journée. Le rythme de sécrétion de mélatonine persiste avec une période d'environ 24 h quand les animaux sont laissés en obscurité permanente, ce qui démontre la présence d'un rythme endogène. Le rythme d'activité nerveuse qui commande la sécrétion de mélatonine par la glande pinéale est généré par le noyau supra-chiasmatique (SCN) de l'hypothalamus, et les informations nerveuses atteignent la glande pinéale par l'intermédiaire du système nerveux sympathique. La lumière, perçue par la rétine, synchronise le rythme endogène avec le cycle cicadien de la photopériode externe, et règle la durée de sécrétion de mélatonine nocturne qui est ainsi directement proportionnelle à la durée de la nuit. Les changements de durée de sécrétion nocturne de mélatonine sont critiques pour la transmission de l'information saisonnière. Le site et le mécanisme d'action de la mélatonine dans le cerveau restent largement méconnus. La sécrétion de LH et FSH par l'hypophyse qui contrôle les gonades est régulée par le GnRH sécrété par l'éminence médiane de l'hypothalamus. Les corps cellulaires des neurones qui projettent dans l'éminence médiane sont situés dans l'aire préoptique et la base du télencéphale. L'autoradiographie (mélatonine radioactive) révèle que ses récepteurs sont situés principalement dans des zones distinctes de celles qui contiennent les neurones à GnRH. La mélatonine agit sans doute sur la sécrétion de GnRH par l'intermédiaire d'autres neurones. Les identités neurochimiques de ces systèmes restent inconnues bien que les catécholamines et les opiacés aient été identifiés comme des inhibiteurs de la sécrétion de GnRH, et qu'on porte généralement un intérêt considérable aux neurones glutamatergiques en tant que stimulateurs de la sécrétion de GnRH. Les actions de la mélatonine représentent un défi pour le neurobiologiste, en particulier parce que son action dépend entièrement de son profil de sécrétion plutôt que simplement de sa présence ou absence. Les études sur la mélatonine ont aussi des applications, puisque son utilisation permet de manipuler le saisonnement des animaux d'élevage.

mélatonine / reproduction / GnRH / LH / FSH

NEUROENDOCRINE CONTROL OF REPRODUCTION

Ovarian and testicular function is under the control of gonadotrophin secretion from the pituitary gland. The release of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary is regulated by the secretion of gonadotrophin-releasing hormone (GnRH). In both sexes, the gonads are capable of being activated out of season by appropriate treatment with gonadotrophins (McNeilly *et al,* 1982). Likewise, LH and FSH secretion can be elicited from the pituitary gland by treatment with exogenous GnRH during the non breeding season, thus reproductive inactivity ultimately stems from inadequate release of GnRH (Lincoln, 1979). This decapeptide is secreted from neuron terminals in the median eminence of the hypothalamus into the capillaries of the hypothalamic portal system, and is thus delivered to the pituitary gland. Studies in the reproductively active sheep in which portal blood has been collected reveal that this peptide is released in an episodic manner, rather than being secreted continuously, and that each pulse of GnRH secretion elicits a pulse of LH secretion from the pituitary gland (Caraty and Locatelli, 1988; Moenter *et al,* 1990). The frequency of LH pulses in the sheep is very much reduced in the non-breeding season, presumably reflect-

ing a reduced frequency of episodic GnRH secretion. No difference in the number or form of immunocytochemically identified GnRH neurons exists between the breeding season and the non-breeding season, though the perikarya are slightly larger in the reproductively inactive condition (Lehman *et al,* 1986). Correspondingly, the amount of GnRH stored in the hypothalamus is greater in reproductively inactive sheep (Ebling *et al,* 1987), thus it does not appear that inadequate GnRH synthesis underlies the reproductively inactive state. Moreover, GnRH secretion can be activated in the non-breeding season by treatment with *N*-methyl-D-aspartate, an agonist for the excitatory neurotransmitter glutamate (Ebling and Foster, 1990; Jansen *et al,* 1991), which demonstrates that GnRH neurons are capable of secretion in the non-breeding season. Thus, it is concluded that seasonal infertility in the sheep results from changes in the activity of afferent neurons which control GnRH secretion. In species in which seasonal reproduction is brought about by the reactivation of embryonic development, secretion of prolactin may be the key neuroendocrine event rather than changes in GnRH secretion (see article by Martinet, this issue).

THE ROLE OF THE PINEAL GLAND

Although the identity of neurons which initially respond to changes in photoperiod is unknown, much is known about the mechanism by which photoperiod is transduced into a neuroendocrine signal. Many experiments in the last 30 yr have demonstrated the pivotal role of the pineal gland in mammalian photoperiodism. Removal of the pineal gland, or destruction of the neural inputs to it, prevents seasonal responses to photoperiod. Interestingly, early studies in pinealectomized sheep maintained outdoors on pasture failed to reveal its role (Roche *et al,* 1970), because as subsequent studies have shown, in the absence of photoperiodic information, the sheep can still respond to social cues from intact male or female sheep (Wayne *et al,* 1989). This illustrates the importance of secondary cues in addition to photoperiod in regulating seasonal reproduction. Under experimental conditions where animals are housed under artificial light and isolated from other seasonal cues, the effect of removing the pineal is dramatic. For example, in the Syrian hamster, transfer from long to short photoperiods induces rapid testicular regression or anoestrus. However, animals pinealectomized before the change in daylength remain reproductively active (Goldman and Darrow, 1983).

MELATONIN AS NEUROCHEMICAL DARKNESS

It is now clear that the pineal indoleamine melatonin, first isolated by Lerner in 1958, provides a chemical representation of darkness. The pineal gland, however, does not respond directly to light. It receives information *via* the retina which is processed by the suprachiasmatic nucleus (SCN) in the hypothalamus before reaching the pineal *via* sympathetic innervation. Melatonin secretion is an example of an endogenous circadian rhythm; thus it persists with a periodicity of $\approx$ 24 h in animals maintained in constant darkness (Lincoln *et al,* 1985). The rhythmic neural signal which drives pineal secretion is generated within the SCN. The environmental light-dark cycle serves to synchronize this rhythm of secretion, and light also directly suppresses melatonin secretion. A combination of these 2 effects ensures that the nocturnal period of melatonin secretion varies in direct proportion to the length of the night. Although the pineal gland may secrete many indoleamines and neuropep-

tides, treatment of pinealectomized animals with appropriate patterns of melatonin reproduces the seasonal effects of photoperiod, indicating that melatonin is the key pineal hormone involved in transducing photoperiodic information. In the example cited above, treatment of pinealectomized Syrian hamsters with 10 h of melatonin per d, to mimic the pattern of secretion normally observed on short days, will induce testicular regression (Maywood *et al,* 1991). Studies in many other species including rodents, sheep and deer have demonstrated that melatonin can influence a wide range of body systems known to change seasonally, including prolactin secretion, wool, horn and antler growth and fat metabolism (Lincoln and Ebling, 1985).

One contemporary area of research is investigating how light adjusts the SCN clock which drives melatonin secretion, using the expression of immediate early genes as a marker of those neurons which have been activated by light. This approach suggests that glutamate may be a neurotransmitter which conveys the effects of light to the SCN (Colwell *et al,* 1991; Ebling *et al,* 1991). A second area of interest is to understand which features of the melatonin signal actually convey photoperiodic information. Traditionally, endocrinologists have been able simply to measure the concentration of a given hormone in order to predict its action. This is not the case with melatonin, where it is clear that the daily pattern of secretion is critical. The same amount of melatonin administered over 10 h a day to a pinealectomized hamster which would induce gonadal regression would have the opposite effect if it were given over a period of 4 h per day, in which case it would maintain gonadal function or induce testicular recrudescence in a reproductively inactive individual (Carter and Goldman, 1983). Seasonal changes in the amplitude of the nocturnal peak, in the duration of the nightly rise, and in the phase of the melatonin rise relative to the light–dark cycle have all been proposed as mechanisms by which melatonin could convey information (Reiter, 1987). The majority of the evidence supports the view that duration is the key parameter. For example, timed infusions of different durations to sheep or hamsters lacking endogenous melatonin secretion induce photoperiodic responses regardless of the actual time of day that they are given, indicating that the phase of the melatonin rhythm relative to the ambient light dark cycle is not critical (Wayne *et al,* 1988; Maywood *et al,* 1990).

SITES OF ACTION OF MELATONIN

The site and mechanism of action of melatonin within the brain is an area of active interest. The secretion of luteinizing hormone and follicle stimulating hormone from the pituitary gland which controls gonadal function is regulated by secretion of gonadotrophin releasing hormone (GnRH) from the median eminence of the hypothalamus. The cell bodies of the GnRH neurons which project to the median eminence are located in the preoptic area and basal forebrain. Autoradiography using radiolabelled melatonin (^{125}iodomelatonin) reveals that binding occurs mainly in areas that are separate from those containing GnRH neurons. The pattern of ^{125}iodomelatonin binding varies considerably from species to species. In the sheep many areas of the hypothalamus, basal forebrain, thalamus and hippocampus bind melatonin (Bittman and Weaver, 1990). The pattern is more restricted in rodents, though binding is present in the ventromedial and suprachiasmatic nuclei of the hypothalamus, the preoptic area and the paraventricular nucleus in the midline of the thalamus (Weaver *et al,* 1989), whereas in mustelids no binding has been detected with

^{125}iodomelatonin within the central nervous system (Weaver and Reppert, 1990). One common feature of all the species studied so far has been the presence of considerable binding in the pars tuberalis of the pituitary gland.

These autoradiographical studies can provide evidence for the location of melatonin receptors, but they do not demonstrate that binding occurring in any particular area has a function in regulating seasonal responses. Several experimental approaches have been adopted to investigate which areas are of functional importance. One approach has been to block the action of melatonin by imunisation. In general, active immunisation or systemic passive immunisation have failed to influence photoperiodic responses (Arendt *et al,* 1981), whereas intraventricular injection of an anti-melatonin serum blocks the inhibitory effect of short days on FSH secretion in hamsters (Bonnefond *et al,* 1989). In the former studies, it seems unlikely that melatonin antibodies in the systemic circulation would have access to intracranial areas. Thus, collectively the studies indicate that melatonin acts within the central nervous system to influence seasonal reproduction. Although pharmacological antagonists have been developed which block *in vitro* actions of melatonin, the seasonal effects of melatonin have not yet been prevented by such compounds, thus this approach remains to be exploited (Lincoln and Kelly, 1989). The complementary approach has been to administer melatonin locally within the brain. A seasonal response to a low dose intracranial melatonin treatment has been demonstrated in several species (Glass, 1984; Hastings *et al,* 1988). In general, these studies indicate a site of action within the medial hypothalamus because implants in extrahypothalamic areas are ineffective in altering seasonal reproduction, but as with the immunological approach, this technique lacks precision because diffusion of melatonin away from the site of delivery cannot be readily controlled or measured. A third approach has been to lesion discrete areas of the brain and to determine whether responses to a change in photoperiod or to melatonin treatment can occur. This technique has gained fresh impetus from the autoradiographic identification of melatonin binding sites. However, although anatomical precision is better than the other approaches, interpretation of lesion studies is difficult. For example, lesions of the anterior hypothalamic area prevent melatonin suppression of FSH in castrated Syrian hamsters (Bonnefond *et al,* 1989). However, melatonin receptors have not been localized in this region, so this area probably has a permissive role for the melatonin response rather than being the actual site of melatonin action. Although each technique individually has certain problems of interpretation, collectively they provide good evidence for a site of action in the brain within the medial hypothalamus.

CELLULAR ACTIONS OF MELATONIN

The recent identification of a high density of melatonin binding sites in the pars tuberalis of the pituitary gland has led to the use of this tissue for investigating the cellular mechanism of action of melatonin. Melatonin inhibits forskolin-stimulated cyclic AMP production and protein kinase A activity in primary cultures of pars tuberalis cells, *via* a G-protein coupled receptor (Morgan *et al,* 1989, 1990). Current studies are attempting to determine whether the activity of intracellular phosphoproteins changes as a result. It should be borne in mind that the biochemical responses characterised to date are acute; it remains to be determined how they relate to the complex responses of neural tissues to timed exposure to melatonin.

CONTROL OF GnRH SECRETION

The spatial dissimilarities between melatonin binding and action revealed by autoradiographic and experimental studies and the distribution of GnRH neurons suggest that the actions of melatonin are likely to be mediated by interneurons, rather than being directly on GnRH neurons. Pharmacological studies in which animals are treated with antagonists for different neurotransmitter systems have revealed that both catecholamine (Meyer and Goodman, 1985) and endogenous opioid systems (Ebling and Lincoln, 1985) inhibit GnRH secretion. Catecholamine inhibition is prevalent in the anoestrous season, and specific lesions of hypothalamic dopaminergic neurons lead to an increase in LH pulse frequency at this time of year in sheep (Thiéry *et al,* 1989). Thus, catecholamine inhibition may contribute to the seasonal inactivation of the reproductive axis. The effects of opioid receptor blockade on LH secretion are more pronounced in the breeding season, thus endogenous opioids may play a greater role in homeostatic feedback in reproductively active animals than in driving seasonal changes in reproductive function (Lincoln *et al,* 1987). Currently there is considerable interest in the role of excitatory amino acid neurotransmitters (*eg* glutamate) as stimulatory inputs for GnRH secretion. As previously noted, glutamate agonists stimulate LH secretion during the non breeding season in sheep (Ebling and Foster, 1990; Jansen *et al,* 1991) and hamsters (Hui *et al,* 1992). Moreover, chronic agonist administration can drive testicular recrudescence in the Syrian hamster (Urbanski, 1990). It is hypothesized that seasonal testicular regression occurs when endogenous glutamatergic stimulation decreases. However, studies with antagonists have yet to be carried out to determine whether experimentally-induced decreases in glutamatergic action can cause testicular regression.

FUTURE DIRECTIONS

Clearly, many major questions regarding the site and mechanism of melatonin action action remain unanswered. The pars tuberalis is proving to be a valuable experimental model for understanding the intracellular actions of melatonin, providing evidence that melatonin inhibits the action of adenylate cyclase, though it remains to be determined whether this will also be the case in neural tissues. Attempts to sequence the melatonin receptor should yield better tools for studying the neurochemical identity of melatonin responsive neurons and its intracellular actions. The development of pharmacological agonists and antagonists will not only stimulate research, but could have commercial applications. Already, manipulation with native melatonin provides a means of regulating seasonality under field conditions. Moreover, the actions of melatonin provide a novel challenge for the neuroscientist because its actions are entirely dependent on its pattern of secretion rather than simply its presence or absence. Understanding this seasonal timing system provides an insight into the very nature of biological clocks and time keeping.

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