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Role for Acetylcholine in Mediating Effects of Light on Reproduction

Abstract. *The length of day, or photoperiod, regulates the annual cycle of reproductive activity in the golden hamster. The inhibitory effects of a short-day photoperiod on testicular function were prevented by nighttime, but not daytime, intraventricular injections of carbachol, a cholinergic agonist. Short pulses of light during the night also block short-day induced testicular regression. The findings suggest that acetylcholine may play an important role in the mechanism through which information about the light-dark environment is transferred to the hypothalamic-pituitary-gonadal axis.*

In many avian and mammalian species the effects of seasonal changes in day length on neuroendocrine-gonadal activity are mediated by an internal 24-hour biological clock (*1, 2*). Although there is a clear relation between the biological clock and the hypothalamic-pituitary-gonadal axis, little is known about the neurochemical events underlying the effects of light on clock-controlled changes in reproductive function. Acetylcholine has been implicated in mediating the effects of light on the circadian system by reports that carbachol, a cholinergic agonist, mimics the phase-shifting effects of light on both the circadian rhythms of wheel-running activity in mice and pineal serotonin *N*-acetyltransferase activity in rats (*3*).

To identify the neurotransmitters involved in the circadian-based photoperiodic control of reproduction, we focused on a possible role for acetylcholine. We used male golden hamsters (*Mesocricetus auratus*) because maintenance of gonadal function in these animals does not require daily prolonged periods of photostimulation (*4, 5*). Testicular regression occurs in hamsters exposed to nonstimulatory short days, but can be prevented by exposing them to as little as 1 second of light near the middle of the night either every day or every other day (*6*). We sought to determine whether the administration of carbachol during the night could mimic the effects of short pulses of light on gonadal function in hamsters transferred from a stimulatory light-dark (LD) cycle of 14 hours of light and 10 of darkness to a nonstimulatory LD 6:18.

Twenty-eight adult male golden hamsters (*7*) that had been housed five to six

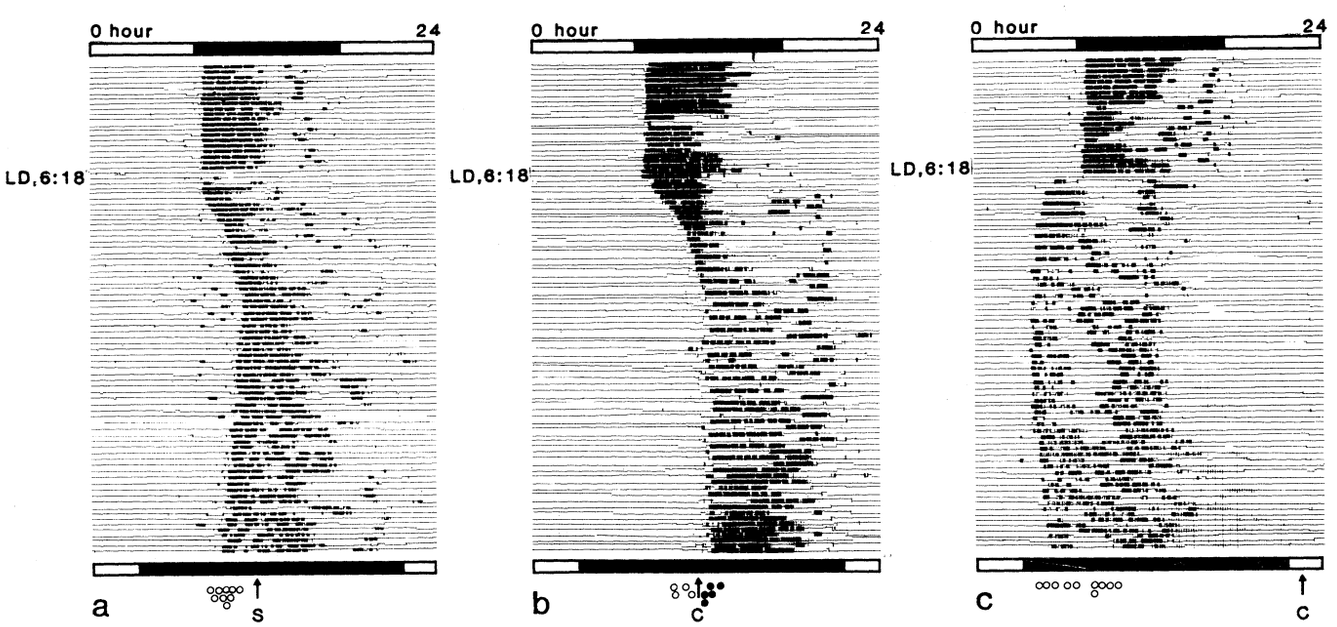

Fig. 1. The activity records of three individual hamsters that were first exposed to LD 14:10 and then transferred to LD 6:18 on the day indicated. LD 14:10 is illustrated at the top and LD 6:18 at the bottom of the activity charts, with the time of darkness represented by the black bars. (a) The activity of an animal injected every other day with saline (*S*) 8 to 8.5 hours after lights-off (time of injections represented by arrow). (b) The activity of an animal injected with carbachol (*C*) 8 to 8.5 hours after lights-off. (c) The activity of an animal injected with carbachol 1 to 1.5 hours after lights-on. The mean (± S.E.M.) activity onsets for animals in groups 1, 2, and 3 occurred 5.9 ± 0.2, 8.1 ± 0.4, and 3.9 ± 0.6 hours after lights-off, respectively. The circles below the activity records depict the time of the onset of activity relative to LD 6:18 for individual animals in the three groups of animals. Open circles represent animals with small testes (paired testis weight < 1000 mg); closed circles represent animals with large testes (paired testis weight > 1000 mg) at the end of the experiment.

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per cage and maintained on LD 14:10 were transferred to individual cages equipped with a running wheel, where circadian rhythm of locomotor activity could be recorded (*8*). The animals remained on LD 14:10, and in 3 to 5 weeks a stainless steel cannula was stereotaxically placed into the right lateral ventricle to allow administration of saline or carbachol directly into the cerebrospinal fluid (*9*). About 5 weeks after the transfer to individual cages, the width of the right testis was measured to the nearest 0.1 mm for each animal (*10*), and all the animals were then put on LD 6:18. The animals were randomly divided into three groups and received intraventricular injections of either saline or carbachol (*11*) every other day according to one of the following protocols: group 1 ($N = 9$) received saline injections 8 to 8.5 hours after the lights-off, group 2 ($N = 9$) received carbachol 8 to 8.5 hours after the lights-off, and group 3 ($N = 10$) received carbachol 1 to 1.5 hours after lights-on. After 10 weeks, the animals were anesthetized, and the testes were removed and weighed. The animals were then perfused with 0.9 percent saline, followed by 10 percent Formalin, and the brains removed for verification of the placement of the cannula (*12*).

Hamsters maintained on LD 14:10 begin running on the wheel near the time of lights-off, but those entrained to LD 6:18 begin 4 to 7 hours after lights-off (*4, 13*). The nine animals that we injected with saline at night began their activity 5 to 7 hours after lights-off once entrained to LD 6:18 (Fig. 1a). After 10 weeks on LD 6:18, complete testicular regression was observed in all animals in group 1 (Fig. 2). Injections of carbachol at night had a statistically significant effect both in delaying the onset of activity ($P < .01$, $U = 79$) and preventing testicular regression ($P < .01$, $U = 71$) relative to saline-injected control animals. Also, a striking correlation was observed between the onset of activity and the effects of carbachol on testicular weight. In five animals injected with carbachol at night (group 2), the onset of activity occurred after the injection of carbachol (Fig. 1b) and the inhibitory effects of short days on testicular function were either totally or partially blocked (Fig. 2). In the four other animals in group 2, in which the onset of activity occurred at the same time or before the injection of carbachol, complete testicular regression was observed. Injections of carbachol during the light phase had no effect on short-day induced testicular regression (Fig. 2), but the onset of activity

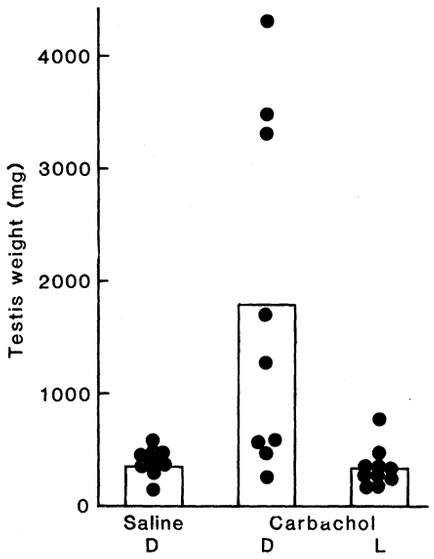

Fig. 2. Mean paired testis weight (bars) for three groups of male hamsters that were exposed to LD 6:18 for 10 weeks. Group 1 was injected with saline 8 to 8.5 hours after lights-off (*D*) and groups 2 and 3 with carbachol, either 8 to 8.5 hours after lights-off (*D*) or 1 to 1.5 hours after lights-on (*L*), respectively. The mean (± S.E.M.) paired testis weights of animals in groups 1, 2, and 3 were 359.0 ± 36.3, 1764.7 ± 510.2, and 328.7 ± 54.8 mg, respectively. Closed circles represent values for individual animals.

was phase-advanced in five of the ten animals in group 3 relative to the saline-injected control animals (Fig. 1c).

Because injections of carbachol 8 hours after lights-off had an effect on both activity and testicular function in some animals that was similar to that of a 1-second pulse of light at this time (*6*), our results suggest that nighttime injections of carbachol may mimic the effects of light on neuroendocrine-gonadal function. The lack of uniform responses to the nighttime injections may be related to the fact that we are near the threshold for the induction of a response to carbachol. Daytime injections did not influence short day–induced testicular regression, indicating that carbachol does not have a nonspecific effect on the hypothalamic-pituitary-gonadal axis. Since nighttime injections of carbachol alter gonadal response to short days as well as the entrainment of the circadian rhythm of activity, carbachol may be acting on the circadian system that is involved in the photic control of reproduction and the rhythm of activity. Such an action is supported by the finding that daytime injections of carbachol (group 3) caused five animals to become active before the usual time for hamsters entrained to LD 6:18 (Fig. 1). These daytime injections coincided with the early part of the hamsters' subjective day, a time when light is known to induce phase advances of the activity rhythm (*14*). Perhaps carbachol stimulates the circadian system to a greater degree than light alone.

The results of a number of experiments indicate that light induces a photoperiodic response only when it is coincident with a particular phase (or set of phase points) of one or more circadian rhythms (*2*). In the hamster, this sensitive phase to light appears to begin near the onset of activity and to last for 11 to 12 hours (*5*). Our finding that an injection of carbachol (or a 1-second pulse of light) 8 hours after lights-off can partially or totally prevent testicular regression in hamsters exposed to LD 6:18 is subject to two interpretations: the carbachol, in mimicking the effects of light, was injected during the light-sensitive phase; or, the carbachol may be inducing a phase shift in the circadian system such that the 6-hour period of light, which occurs 10 hours after the injection, is now coincident with the later portion of the light-sensitive phase. The two possibilities are of course not mutually exclusive. Daytime injections of carbachol are presumably ineffective in maintaining testicular function because they did not coincide with the sensitive phase to light and/or did not shift the circadian system such that the sensitive phase was coincident with the 6-hour period of light.

Attempts to localize the circadian system in mammals have focused on the suprachiasmatic nucleus (SCN) of the hypothalamus; bilateral destruction of this nucleus results in the abolishment, or severe disruption, of a variety of different circadian rhythms (*15*). The effects of light on the rhythm of activity in the hamster, as well as the effects of light on neuroendocrine-gonadal function, are both thought to be mediated through the SCN (*16*). Whether the effects of carbachol on activity and testicular function that we observed are also mediated by the SCN is not known, but the nuclei do appear to be a likely target since (i) carbachol can mimic the phase-shifting effects of light pulses on free-running circadian rhythms that are regulated by the SCN (*3*), (ii) cholinergic receptors and choline acetyltransferase have been found in the SCN (*17*), and (iii) the firing rate of neurons in the SCN is altered in a similar manner after exposure to either light or the iontophoretic application of acetylcholine to the SCN (*18*).

The effects of carbachol may also be attributed to a direct or indirect action on the pineal gland since this structure plays an important role in the photoperiodic

control of reproduction (*19*) and carbachol can alter pineal enzyme activity (*3*). A direct action of carbachol on the pineal gland appears unlikely because the effects of carbachol on reproductive function occurred concomitantly with an alteration of the circadian rhythm of activity, and the pineal gland does not play a major role in the regulation of the activity rhythm (*20*). An alternative possibility is that carbachol-induced changes in SCN activity lead to an alteration in pineal function and, ultimately, gonadal activity.

Although it has long been recognized that the photoperiod can influence seasonal reproductive cycles in animals, only now are some of the neural events that mediate the effects of light on reproductive activity being elucidated. Our results suggest that acetylcholine may play a key role in the mechanism by which a circadian clock is involved in photoperiodic time measurement. Our experimental approach, in which a discrete stimulus (for example, electrical or chemical) is presented at a specific circadian time to induce a photoperiodic response, may prove valuable in determining the neurochemical and neurophysiological events that enable an organism to measure the seasonal change in day length.

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7. The hamsters [Lak:LVG(SYR)] were obtained at 9 weeks of age from Lakeview Hamster Colony, Newfield, N.J.
8. Revolutions of the running wheel were recorded through a microswitch as a pen deflection on an Esterline Angus Event recorder, and daily records kept for each animal.
9. After anesthetizing the hamsters with sodium pentobarbital (6 mg per kilogram of body weight), a circular piece of the skull (2 mm in diameter) was removed and a 22-gauge stainless steel cannula was placed in the brain at the following stereotaxic coordinates with the head held 5 mm above horizontal: 1.6 mm anterior to bregma, 1.5 mm to the right of the midsagittal sinus, and 3.2 mm ventral to the dura mater. Dental cement was applied to the base of the cannula and three anchoring screws in the skull to stabilize the cannula in a fixed position.
10. At this time the testes were large in all animals (mean testis width, 12.2 ± 0.1 mm). The testes of sexually mature hamsters weigh about 3000 mg [F. W. Turek and S. H. Losee, *Biol. Reprod.* **18**, 299 (1978)].
11. A 2-μl injection of either physiological saline or a solution of 0.01*M* carbachol dissolved in saline was injected under light ether anesthesia by a Hamilton microliter syringe. The length of the guide cannula was such that the needle extended 0.5 mm ventral to the tip into the lateral ventricle. A red safelight (Kodak filter 1; 0.5 to 1.0 lux) was used for injections during the dark.
12. Frozen brain sections at a thickness of 60 μm and sections were examined under a light microscope to verify placement of the cannula tip; in all animals, the cannula was observed to extend into the lateral ventricle.
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Physalaemin: An Amphibian Tachykinin in Human Lung Small-Cell Carcinoma

Abstract. *Immunoreactivity to the amphibian peptide physalaemin was characterized from extracts of a human lung small-cell carcinoma by immunological, chemical, and pharmacological means. Tumor-related peptide cross-reacted with three antiserums to physalaemin to yield 1.1 to 1.6 nanomoles per gram of tissue. Physalaemin and tumor peptide had similar retention times on high-performance liquid chromatography after chemical and enzymic modifications that included pH changes, oxone oxidation, use of a hydrophilic ion-pairing reagent, and digestion with trypsin and pyroglutamate aminopeptidase. Both physalaemin and the tumor peptide produced a contractile response of isolated guinea pig ileum at threshold concentrations of approximately 100 to 150 picograms per milliliter. These data suggest that small-cell carcinoma of the lung contains a physalaemin-like peptide that has structural and biological homology to its amphibian counterpart.*

Small-cell carcinoma of the lung (SCCL) exhibits heterogeneity among its constituent cells (*1*), which is reflected in its variable biochemical properties, ectopic (inappropriate) production of peptide hormones (*2, 3*), and chromosomal abnormalities (*4*). Most notable among the hormones detected in extracts of SCCL and at elevated levels in the plasma of patients with this tumor were adrenocorticotropin (*2, 3*), prolactin, oxytocin (*2*), calcitonin, and β–human chorionic gonadotropin (*3*). Immunoreactivity to the amphibian peptide bombesin has been found in all SCCL's resected from patients (*5*) or maintained as tumor-derived cell lines (*6*) or propagated in nude mice (*7*). The immunoreactive substance appeared to have structural homology with the amphibian peptide (*7*) rather than with the larger bombesin-like peptides (*8*). Another amphibian peptide, physalaemin, was detected in SCCL by a radioimmunoassay and by immunohistochemistry (*7*). This group of related peptides (tachykinins) includes substance P and has potent pharmacological effects on extravascular smooth muscle, blood

Table 1. Reverse-phase HPLC retention times of amphibian physalaemin and SCCL physalaemin immunoreactivity. Values are given as means ± standard deviation, with the number of separate experiments in parentheses; N.D., not determined.

Conditions	Retention time (minutes)			
	Physalaemin		Tumor immunoreactivity	
	Oxidized	Reduced	Oxidized	Reduced
HFBA, *p*H 2.3	27.2 ± 0.8 (3)	30.2 ± 0.2 (7)	27.9 ± 0.6 (5)	30.5 ± 0.4 (5)
plus trypsin	33.3*		33.0*	
Acetate, *p*H 4.2	29.5	32.8 ± 0.1 (2)	29.5 ± 0.1 (2)	32.7 ± 0.1 (2)
Phosphate, *p*H 7.0	N.D.	33.1 ± 0.1 (2)	29.4	33.1

*The tryptic fragment lacks a thio-containing residue and hence is neither oxidized nor reduced.

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