Evidence for both local and central regulation of rat rod outer segment disc shedding

Paul S. Teirstein, Arnold I. Goldman,* and Paul J. O'Brien

Rats entrained to a 7 A.M.:7 P.M. lighting cycle had one eye patched and then were placed in either constant light or in a 2 A.M.:2 P.M. lighting cycle. In both cases, the patched eyes maintained the original pattern of rod outer segment (ROS) disc shedding, whereas the open eyes responded to the ambient lighting conditions. When the optic nerves were severed at the optic chiasm, the shedding rhythm persisted, but it was no longer possible to shift the rhythm to a new lighting cycle. From these experiments we conclude that ROS disc shedding is regulated both by local oscillators within the orbit as well as by central elements.

Key words: photoreceptor renewal, circadian rhythm, phagocytosis, shedding, outer segment, circadian oscillator

Vertebrate rod outer segments (ROSs) are continually renewed by the addition of newly synthesized discs at the outer segment base and the shedding of old discs at the tip.1-4 The shed discs are phagocyted by the retinal pigment epithelium (PE) where they appear as heavily staining inclusion bodies termed phagosomes.3>5 When rats are maintained in cyclic light the concentration of phagosomes in the PE varies throughout the day.6-10 Soon after the lights are turned on, there is a burst of synchronous disc shedding resulting in an abundance of large phagosomes in the PE. During the first 5 hr of light the number of phagosomes is three to four times that observed during the remainder of the light cycle. This cyclic event can be characterized as circadian in nature (for a review of circadian rhythms see refs. 11 to 14), since it will continue in synchrony under conditions of constant darkness.6-10 A 24 hr period of constant light (LL) has been shown to inhibit the shedding rhythm.10 Furthermore, the phase of the rhythm can be advanced or delayed by modification of the lighting regimen.10 There are various circadian rhythms documented in the rat, ranging from activity rhythms11-14 to rhythms in basal temperature and hormone concentration.11-18 As yet, investigators have been unable to find a link between these other rhythms and the rhythm of disc shedding. Neither pinealectomy, superior cervical ganglionectomy, and hypophysectomy nor thyroidectomy and parathyroidectomy altered disc shedding. In light of these findings a more general question can be posed. Is the rhythm of disc shedding controlled by factors external to the eye, or is this rhythm regulated locally within the eye? This paper will provide evidence to
suggest a complex regulatory system with both central and local control mechanisms. Preliminary accounts of this work have already been published.\textsuperscript{19, 20} \textbf{Materials and methods} All the animals used in this study were male Sprague-Dawley rats 6 to 9 weeks of age obtained from Zivic-Miller Laboratories, Allison Park, Pa. Animals were raised and maintained under conditions of controlled lighting as previously described.\textsuperscript{10} For the experiments requiring ocular occlusion, the animals were anesthetized with 35% chloral hydrate (0.1 ml/100 gm) administered intraperitoneally, and one eyelid from each experimental animal was sutured shut, darkened with India ink, and then patched with black tape and epoxy adhesive. Animals receiving prechiasmatic optic nerve section (ONS) were supplied by Zivic-Miller Laboratories as previously described.\textsuperscript{10} The eyes were processed, and the phagosomes counted by our usual techniques.\textsuperscript{10} One eye per animal was used, and 6 to 10 180 μm fields from each of four to six retinas were used for phagosome counts. In all cases data are expressed as phagosomes per 180 μm ± S.E.M. \textbf{Results} \textbf{Effect of LL on monocularly occluded rats.} To determine whether LL affects each retina independently, rats entrained to a 7 A.M.:7 P.M. light cycle were monocularly occluded and maintained on that cycle. On either the third or fourth postoperative day the animals were placed in low-level LL (5 to 8 ft-cd)\textsuperscript{10} for 24 to 35 hr and were sacrificed in the light. Cyclic shedding was abolished in the eyes that remained open to the light; the number of phagosomes remained at basal levels at all times observed. These results were equivalent to those obtained with the eyes of normal unoperated rats exposed to LL.\textsuperscript{10} The patched eyes, however, resisted the inhibitory effects of LL (Fig. 1). There was a peak of disc shedding at 8:15 A.M., corresponding to a level of 22.5 ± 5.4 phagosomes, which declined slowly and approached basal levels by the afternoon. These eyes had been shielded from any direct effect of LL; instead they experienced constant darkness for the duration of the experiment. When compared to rats placed in cyclic light this peak was lower in amplitude and was broadened. This damped pattern was very similar to that obtained with unoperated rats placed in constant darkness for 5 days.\textsuperscript{10} Thus the patched eye, shielded from the direct effect of LL, continued to shed cyclically. This demonstrates that the inhibitory effect of LL can be exerted within one eye without affecting the other eye. A similar effect has been seen in the frog,\textsuperscript{21} an animal without circadian control of shedding. These results suggest two possible mechanisms for the inhibitory effect of LL. (1) LL acts locally within the orbit to inhibit shedding, or (2) LL acts via the optic nerve system.
Effect of a shift in the lighting cycle on shedding in rats with one eye patched. Animals were raised on a 7 A.M.:7 P.M. light cycle, one eye was occluded, and the animals were placed in a 2 A.M.:2 P.M. light cycle for 5 days. On the sixth day the lights remained off, and the animals were sacrificed at the indicated times. Solid line, Eyes of unoperated animals placed in the same lighting conditions; dashed line, open eyes; alternating line, patched eyes of the experimental animals.

Fig. 2. Effect of LL on ONS rats. To distinguish whether LL acts locally or via the optic nerves to inhibit rhythms generated by two independent oscillators.

Effect of LL on ONS rats. To distinguish whether LL acts locally or via the optic nerves to inhibit the shedding rhythm, animals raised on the 7 A.M.:7 P.M. light cycle were subjected to bilateral ONS. This procedure surgically separated the orbit from the central nervous system (CNS) without interfering with the retinal blood supply. Rats subjected to this procedure exhibited no histological evidence of retinal degeneration and continued to shed cyclically 14 days after surgery (Table I). Seven days after surgery these animals and a group of sham-operated control animals were placed in extended LL. At 5:15 A.M. after 25% hr of LL, the levels of shedding were 10.0 ± 3.2 phagosomes in experimental and 8.4 ± 1.4 in sham-operated animals, both values at baseline levels. This indicates that LL need not utilize the optic nerves to inhibit disc shedding. Instead, this inhibitory effect is exerted locally within the eyes, each with its own oscillator.

Effect of shift in light cycle on monocularly occluded rats. An important question, however, remained unanswered. Does LL act locally to inhibit a locally induced rhythm, or does LL act locally to inhibit a centrally induced rhythm?

If a central synchronizer were responsible for the regulation of circadian disc shedding, its effects would be mediated either humorally (via a circulating factor) or via discrete neural pathways. To test for the presence of a circulating regulatory factor, we attempted to establish a condition under which the two eyes of a single animal could express two independent circadian rhythms.

One group of rats was prepared with one eye occluded as already described. A second group of rats served as controls. Both groups of animals were subjected to an altered lighting schedule. On this new schedule, the lights were turned on at 2:00 A.M. and off at 2:00 P.M., both times 5 hr earlier than usual. All the animals were placed on the new light schedule for 5 days. On day 6, the day of sacrifice, the animals were kept in constant darkness to ensure that any peaks observed would represent circadian rather than light-triggered shedding.

In the unoperated control rats we found a peak of shedding at 5:45 A.M. (Fig. 2). This peak was 2½ hr earlier than that observed in rats raised on the original light schedule. In addition, the total time of increased shedding was lengthened, extending from 3:45 A.M. to 9:30 A.M., a 5½ hr time period. These results clearly indicate that the pattern of shedding has been altered after 5 days in the new light cycle and are consistent with previous experiments of this type.

In the experimental animals the eyes that remained open to light exhibited a pattern of shedding quite similar to that of the control eyes (Fig. 2). There was a peak at 5:45 A.M., a shift of 2½ hr. The patched eyes, however, resisted the effects of the altered light schedule. Very few phagosomes were seen at 5:45 A.M.; instead, there was a peak at 8:15 A.M.

The different shedding patterns observed in the open and the patched eyes were accompanied by differences in the magnitude of the shedding peak. The mean peak in the open eye was 30.0 ± 1.5, quite similar to that observed in normal rats placed in only 1
Local, central regulation of ROS disc shedding

The mean peak in the closed eye was 22.0 ± 2.1, quite similar to that observed in normal rats after several days of constant darkness.

These results indicate that in a single animal each eye exhibits its own independent circadian rhythm. We were able to establish a new circadian rhythm in one eye without affecting the rhythm of the other. Based on this evidence we conclude that a humoral factor, which would be expected to affect both eyes, cannot be responsible for the direct regulation of ROS disc shedding. If a humoral factor were to mediate the phase shift, it would have to act in concert with light and would thus be a permissive regulator that enabled light to reset the retinal oscillator. Alternatively there may be either (1) an entirely autonomous circadian oscillator within each orbit or (2) two distinct central circadian synchronizers, each transmitting information to a single retinal oscillator via independent neural pathways.

Effect of shift in light cycle on ONS rats. To distinguish whether the shedding rhythm is synchronized locally or centrally, the following experiments were performed. First, a group of animals raised on the 7 A.M.:7 P.M. light cycle was subjected to bilateral ONS as described above. Animals were kept up to 14 days after surgery and then sacrificed in the dark to avoid light-triggered shedding events and thus measure only circadian shedding. Two days after surgery, the 8:15 A.M. phagosome levels were 29.3 ± 6.3, whereas by 14 days after surgery the 8:15 A.M. level had declined to 16.9 ± 2.3 (Table I). Furthermore, there was no significant difference in shedding in animals maintained in cyclic lighting or in darkness for 1 week after ONS.

It appears therefore that although circadian shedding persisted after ONS, it diminished somewhat after the second postoperative day. Interaction with the CNS may be necessary to synchronize the sharp early morning peak of phagocytosis. In addition, this peak diminished to the same extent whether or not the animal was maintained in cyclic lighting.

Another group of rats raised on the 7 A.M.:7 P.M. light cycle was subjected to bilateral ONS. Two to 3 days after surgery one group of these animals was placed on a 2 A.M.:2 P.M. lighting schedule for 5 days. On day 6, all animals were kept in constant darkness and sampled at either 5:00 A.M. or 8:15 A.M.

Control animals on the usual 7 A.M.:7 P.M. light cycle demonstrated a burst of shedding at 8:15 A.M. with lower levels found at 5:00 A.M. (Fig. 3). ONS animals also demonstrated a burst of shedding at 8:15 A.M. This number was attenuated, however, because these animals were sacrificed 8 to 9 days after ONS.

Control animals on the shifted cycle exhibited a high level of phagocytosis at 5:00 A.M., indicating a re-entrainment of their circadian rhythm. ONS animals subjected to the same shifted light schedule, however, failed to demonstrate a corresponding shift in the shedding rhythm. This group exhibited only baseline shedding at 5:00 A.M., whereas increased shedding occurred at 8:15 A.M. Both groups of ONS animals exhibited similar shedding patterns. Baseline shedding is depicted in the 5 P.M. measurements.

Bilateral ONS therefore prevents the re-entrainment of ROS disc shedding. Operated animals subjected to a new altered light
Fig. 4. Proposed model of control of shedding. Light is received by photoreceptor ROSs which send neural information to a "synchronizer" in the CNS. This synchronizer sends a signal to the retinal oscillator which entrains this oscillator to the ambient light cycle. The retinal oscillator in turn initiates a series of dark reactions requiring a minimum of 2 hr of darkness that trigger the shedding of the ROS tip.

schedule retain the old, previously entrained shedding rhythm.

Discussion

The results indicate that the shedding phenomenon can occur in each retina independently, that LL can override the shedding signal locally within a single eye, and that each eye possesses an endogenous oscillator which controls the shedding rhythm.

The results also indicate a role for the CNS in both the re-entrainment and synchronization of circadian ROS disc shedding. We have ruled out direct humoral regulation of shedding. However, we have not conclusively eliminated the possibility of indirect humoral mediation of the CNS control of phase shifts. In the patched eye shift experiments, a humoral factor, if produced by the CNS, might not have effected a phase shift in the patched eye because an entraining light signal could not reach that eye. Although both eyes would have had access to such a permissive regulating factor, only the open eye had access to the shifted light signals. Alternatively, the CNS might carry its regulatory information independently to each retina via neurons. In that case, it is clear that there would have to be separate CNS elements mediating the entrainment of each retinal oscillator.

Various neural pathways are available for CNS regulation of disc shedding. Conflicting evidence exists as to the presence of efferents in the optic nerve.22 Our results are consistent with the existence of efferent nerves through which impulses from the CNS could reach the retina.

The ciliary body is innervated by both parasympathetic and sympathetic fibers. The possibility exists that shedding events in the retina are regulated by efferent impulses from these distant neurons. Any such regulation would be extremely complicated, however, as one would not expect neurotransmitter released at the ciliary body to bind receptors at the anatomically distant retina.

At present, therefore, our evidence indicates that rhythmic disc shedding is maintained by autonomous oscillators located in each orbit. Re-entrainment and synchronization of this rhythm require the presence of intact optic nerves and interaction with the CNS. Other studies have demonstrated that synchronous shedding requires a minimum dark period of 2 hr. Furthermore, a 2 hr pulse of darkness was able to effect a shedding burst in animals with previous ONS, suggesting a retinal location for the dark reaction.10 Our proposed model of how ROS shedding in the rat is controlled is summarized in Fig. 4. The photoreceptor sends information on ambient lighting via the optic nerves to the CNS. Here, independent "synchronizers" (possibly the pacemakers of the rat's circadian system) send synchronizing signals to the retinal oscillators, entraining them to the ambient light cycle. The retinal oscillators initiate a train of events requiring at least 2 hr of darkness for completion,10 which in turn triggers the shedding of the ROS tip.

We thank Adam Atkins for fruitful discussions. We are also grateful for the help and cooperation of Robert Ritch, Kathryn Pokorny, and Steven Podos.

10
REFERENCES