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Research report

Rhythmic expression of clock-controlled genes in retinal photoreceptors is sensitive to 18-beta-glycyrrhetnic acid and 18-alpha-glycyrrhetnic acid-3-hemisuccinate

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Abstract

Chicken retina contains circadian oscillators that drive rhythmic transcription of several genes expressed in photoreceptor cells. To determine if gap junctions assist in coordinating these transcript rhythms, we examined the effects of two compounds, 18α-glycyrrhetnic acid-3-hemisuccinate (ACO) and 18β-glycyrrhetnic acid (18β-GA), on photoreceptor iodopsin and arylalkylamine *N*-acetyltransferase (AANAT) transcript rhythms in embryonic chicken retinal explant cultures that were maintained under different lighting conditions. Both compounds, whose actions include reversibly block gap junction permeability, produced rapid and sustained reductions in iodopsin and AANAT mRNA levels, but did not alter the levels of guanylate cyclase activating protein-1 (GCAP1) mRNA, a noncircadian-regulated, photoreceptor-specific gene. The iodopsin and AANAT mRNA rhythms re-emerged in the cultured retinas within 24 h of removal of the compounds. These results show that the effects of ACO and 18β-GA on iodopsin and AANAT mRNA levels were not due to generalized suppression of gene transcription. The dramatic reduction in the levels of iodopsin and AANAT mRNA induced by these compounds suggests a mechanism of action that directly affects the synthesis and/or degradation of these transcripts rather than the synchronization or function of the retinal oscillators that drive transcription of these genes.
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Theme: Sensory systems
Topic: Retina and photoreceptors

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1. Introduction

Vertebrate retinas contain autonomous circadian oscillators that control the rhythmic expression of several genes in photoreceptor cells (for reviews, see Refs. [6,14]). Pinpointing the locations of these oscillators in retina has proven difficult. Recent studies of transgenic *Xenopus* retina provide direct evidence that the photoreceptors themselves contain autonomous, functional oscillators [13]. Experimental results from retinal dispersed cultures also suggest that cone photoreceptors in chicken retina contain functional oscillators [19]. While consensus is growing that photoreceptors are able to function as autonomous clock cells, it is unclear if the circadian clocks exist in all or only a subpopulation of photoreceptor cells. In addition to photoreceptors, it is also possible that the oscillators regulating gene expression in photoreceptor cells are located in other retinal cells, based on the observation that several core clock proteins are expressed throughout the vertebrate retina with highest levels in photoreceptor and ganglion cells [1,9,12,28,29]. Regardless of where these oscillators are located, it is clear that mechanisms must be present to coordinate the output of

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these oscillators. The search for clock cells in retina has generated significant interest; however, the mechanisms that coordinate circadian rhythms across retinal cell populations have received relatively little attention.

Recent studies of another clock-containing neural tissue, the suprachiasmatic nuclei (SCN), provide insight into mechanisms that coordinate the rhythms generated by ensembles of autonomous oscillators. Gap junction channels that allow the passage of small ions, signaling molecules and low molecular weight metabolites between cells [11] have been shown to assist in the coordination of the rhythmic activities of SCN cells. The permeability of gap junctions in the SCN is controlled by circadian oscillators and the coupling rhythms between the cells coincide with their activity rhythms such that maximum coupling occurs during the day when the cells generate synchronous bursts of neural activity and minimum coupling occurs at night when the cells are electrically silent [8]. Importantly, when SCN slice cultures were treated with either octanol or halothane, two drugs that alter gap junction permeability, arginine vasopressin (AVP) and vasoactive intestinal polypeptide (VIP) secretion rhythms were temporarily lost and re-emerged following removal of these agents [24]. These observations highlight the importance of gap junction channels in maintaining circadian rhythmicity in the SCN.

Gap junctions could also play a role in the synchronization of rhythms in retina. Extensive gap junction networks are present in developing chicken retina as early as embryonic day 7 [2,7]. Prior to synaptogenesis, these junctions assist in the propagation of transient Ca^{2+} waves across the developing chicken retina [7]. To determine if, as in SCN, the gap junction network in chicken retina assists in coordinating photoreceptor iodopsin and AANAT transcript rhythms, we examined the effects of 18α-glycyrrhetinic acid-3-hemisuccinate (ACO) and 18β-glycyrrhetinic acids (18-βGA) on iodopsin and AANAT transcript rhythms in explant cultures maintained under different lighting conditions. Both of these compounds are derivatives of glycyrrhetinic acid. ACO is considered to have the least side effects among the gap junction blockers and has been shown to effectively and reversibly block gap junction channels containing connexin 26 (Cx26) [16], Cx32 [25], and Cx43 [10], the three major connexins expressed in developing chicken retina [3]. However, ACO has recently been reported to reduce voltage-gated Ca^{2+} channel conductance in retina [26] and several intrinsic properties of neurons including action potential threshold and firing rate in response to depolarizing stimuli [21]. The gap junction blocker, 18-βGA, has been shown to block Cl^{-} channels in rat hepatocyte primary cultures [5]. The non-specific effects of these compounds must be considered when interpreting molecular and cellular changes induced by these drugs. In the current study, we hypothesized that these compounds would disrupt the synchronization of the circadian oscillators that drive iodopsin and AANAT mRNA rhythms in the retinal cultures and, as a result, the transcript levels of both genes would assume the average of the peak and trough values of their respective rhythms.

2. Materials and methods

2.1. Chemicals and reagents

The culture media for the explants consisted of Dulbecco's modified Eagle's media (DMEM, Gibco #11995-065) supplemented with 10% fetal bovine serum (FBS) (Hyclone) and antibiotics (130 U/ml penicillin, 130 g/ml streptomycin) (Gibco). The gap junction blockers, 18α-glycyrrhetinic acid-3-hemisuccinate (ACO), 18β-glycyrrhetinic acids (18-βGA), and the chemically-related inactive compound, glycyrrhizic acid (GA), were purchased from Sigma (St. Louis, MO). Stock solutions of ACO (163 mM) and GA (5 mM) were prepared in deionized water. The stock solution of 18-βGA (100 mM) was prepared in dimethyl sulfoxide (DMSO; Sigma). All blockers were used at a final concentration of 100 μM in these experiments, a concentration of ACO that has been shown to effectively block gap junction channels in outer [16,20] and inner retina [22].

2.2. Retinal explant cultures

All experimental procedures were approved by the University of Florida IACUC Committee and were carried out in accordance with the National Institutes of Health guidelines. Fertile White Leghorn chicken eggs (Charles River Laboratories) were incubated on a 12-h light:12-h dark (12L:12D) cycle in incubators illuminated by 20 Watt cool white fluorescent bulbs (90 lx). The lights were turned on at 9:00 AM (Zeitgeber time, ZT0) and were shut off at 9:00 PM (ZT12). Retinal explant cultures were prepared from embryonic day 9 (E9) chickens during the 12-h light period using methods developed in our laboratory [27]. During the first 5 days of culture, all explants were incubated on a 12L:12D cycle at 37 °C in 5% CO2 and were fed every 2 days. The 12L:12D period beginning the day after the cultures were prepared was designated day 1 in vitro (1 DIV).

2.3. Lighting and blocker delivery paradigms

12L:12D—Retinal explant cultures were maintained on a 12L:12D cycle throughout these experiments. Three different blocker delivery schedules were used in these experiments. Cultures treated with ACO or 18β-GA for 48 h received blocker at ZT12 on 4DIV and the blocker was removed at ZT12 on 6DIV. Cultures treated with the blockers for 24 h received them at either ZT12 on 4DIV or ZT0 on 5DIV and the blockers were removed at ZT12 on 5DIV or ZT0 on 6DIV, respectively. Cultures treated with ACO for 6 h received the blocker at either ZT0 or ZT6 on 5DIV and the blocker was removed at either ZT6 or ZT12

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