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The effects of combining serotonin reuptake inhibition and 5-HT$_7$ receptor blockade on circadian rhythm regulation in rodents

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HIGHLIGHTS

- Inhibition of serotonin transporter and 5-HT$_7$ receptor lengthens circadian period.
- Serotonin transporter and 5-HT$_7$ receptor blockade delay phase of circadian activity.
- 5-HT$_7$ receptor activation modulates circadian period length.

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ABSTRACT

Disruption of circadian rhythms may lead to mood disorders. The present study investigated the potential therapeutic utility of combining a 5-HT$_7$ antagonist with a selective serotonin (5-HT) reuptake inhibitor (SSRI), the standard of care in depression, on circadian rhythm regulation. In tissue explants of the suprachiasmatic nucleus (SCN) from PER2::LUC mice genetically modified to report changes in the expression of a key clock protein, the period length of PER2 bioluminescence was shortened in the presence of AS19, a 5-HT$_7$ partial agonist. This reduction was blocked by SB269970, a selective 5-HT$_7$ antagonist. The SSRI, escitalopram, had no effect alone on period length, but a combination with SB269970, yielded significant increases. Dosed in vivo, escitalopram had little impact on the occurrence of activity onsets in rats given access to running wheels, whether the drug was given acutely or sub-chronically. However, preceding the escitalopram treatment with a single acute dose of SB269970 produced robust phase delays, in keeping with the in vitro explant data. Taken together, these findings suggest that the combination of an SSRI and a 5-HT$_7$ receptor antagonist has a greater impact on circadian rhythms than that observed with either agent alone, and that such a multimodal approach may be of therapeutic value in treating patients with poor clock function.

1. Introduction

Virtually all organisms exhibit circadian rhythms. Highly conserved processes are in place to ensure that the timing of daily internal rhythms matches those of the solar day with the primary clock mechanism in mammals originating in the suprachiasmatic nucleus (SCN). So-called retinorecipient neurons receive direct retinal input and dictate the timing of light to an adjacent population of pacemaker cells [1,2]. Other oscillators located within the brain, as well as those distributed widely in the periphery, follow this lead in a highly orchestrated manner [3,4]. The capacity to oscillate is the result of a sequence of transcriptional/translational feedback loops within the oscillator cells with key proteins (PER2, among others) cycling with a period length of 24 h [5–7]. Non-photic input can also serve as zeitgebers or "time-givers" to oscillator function and the SCN also receives input from a number of midbrain and forebrain projections [8,9]. Of these, the serotonergic (5-HT) system is of particular interest in view of its dense innervation of the SCN [10].

While a number of 5-HT receptors have been implicated in SCN function, the 5 HT$_7$ subtype has received a great deal of attention. Evidence for the presence of 5-HT$_7$ receptors in the SCN is clear from anatomical and functional studies [11–16], but the precise nature of their actions is not fully understood [17]. Most experimental work suggests that within the SCN 5-HT$_7$ receptors have a primary role of limiting light-induced changes in the phase of rest:activity rhythms [15,18]. Their function in terms of daily adaptation to the prevailing light:dark cycle is more obscure, although 5-HT$_7$ receptor activation is known to lead to advances in phase [16,19]. What is perhaps clearer is the potential value of the 5-HT$_7$ receptor as a target for novel therapeutic approaches to mental disorders [20]. The concept of 5-HT$_7$ receptor antagonists as antidepressants, antipsychotics, sleep aids and cognitive enhancers has received substantial experimental support [21–24] and, in fact, a 5-HT$_7$ antagonist/SSRI combination has been shown to enhance the increase in cognitive ability observed with the antagonist alone [25]. To the extent that 5-HT$_7$ receptor-mediated modulation of circadian

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rhythm function underlies such clinical utility, a better understanding of its action within the SCN might benefit the field.

Disruptions in circadian rhythm regulation are thought to lead to increased risk of cardiovascular disease, cancer, diabetes and obesity, among other health-related consequences [26–28], and there is a growing link to mood disorders [29]. Perception of mood follows a circadian pattern in healthy individuals and loss of synchrony may lead to maladaptive changes [30]. Unipolar and bipolar depression have been most commonly linked to the circadian clock and a new generation of antidepressants appears poised to take advantage of this connection [31–33]. Perhaps more speculatively, the question arises as to the role of traditional pharmacotherapies — the selective serotonin reuptake inhibitors (SSRIs) and the serotonin norepinephrine reuptake inhibitors (SNRIs) — in modulating circadian rhythm function. Given their current widespread use, there is a growing need to add to the handful of studies that have examined these agents alone or in combination with selective modulators that might fine-tune changes in net 5-HT neurotransmission [34–38].

5-HT_7 receptors represent an interesting target in such multimodal combinations. Selective antagonists of this receptor subtype appear to be robustly active in behavioral measures of antidepressant-like responses when administered with an SSRI [39–41]. A combined 5-HT_7 receptor antagonist/SSRI effect on cortical 5-HT release may underlie these observations, although the nature of this interaction in other brain regions is unknown. A goal of the present investigation was to gain a better understanding of the respective roles of 5-HT_7 receptors and SSRIs in SCN function. To this end, tissue explants from mice genetically modified to provide a bioluminescent measure of mPER2 cycling were used as a measure of oscillator function, as determining the pattern of clock gene expression has proven to be a useful means to monitor changes in circadian period length and amplitude [9,42]. Wheel running activity was used as a means to assess circadian behavior at the whole animal level.

2. Materials and methods

2.1. Animals

Three to six-month-old male homozygous mPER2::LUCIFERASE (PER2::LUC) knock-in mice [43] purchased from Jackson Laboratory, Bar Harbor, ME were housed in a 12 h:12 h light:dark (12:12 LD) cycle for at least 2 weeks prior to dissection and tissue collection. Two-month-old Sprague–Dawley rats purchased from Charles River Laboratories (Wilmington, MA) were housed individually with free access to running wheels (model 80820, Lafayette Instruments, Lafayette, IA) for the duration of the experiment. All animals were maintained under controlled environmental conditions (22 ± 2 °C; lights on at 06:00 to 18:00 h) with food and water available ad libitum. All animal protocols were approved by the Lundbeck Research USA, Inc. Institutional Animal Care and Use Committee.

2.2. Drug treatments

For the in vitro studies, AS19, a reported 5-HT_7 receptor partial agonist [7,39], and SB269970, a 5-HT_7 receptor antagonist [44] (Tocris Bioscience, Ellisville, MO) were dissolved in DMSO and sterile water, respectively; the SSRI escitalopram (H. Lundbeck A/S, DK) was dissolved in sterile water. At the time of tissue treatment, drugs were diluted into the culture media to final concentrations. For the acute and subchronic in vivo treatments, drugs were dissolved as follows: AS19 in 20% β-cyclodextrin (Sigma-Aldrich, St. Louis, MO), and SB267790 and escitalopram in saline or 5% β-cyclodextrin. Prior to administration, pH was adjusted toward pH 5–6 with 0.1 N NaOH; more physiological pH values caused compounds to precipitate.

Drugs and vehicles were administered subcutaneously in a volume of 2 ml/kg body weight to investigate the acute effects of serotonergic modulation, or via osmotic minipumps (ALZET Osmotic Pumps, Cupertino, CA) to investigate phasic effects. Once daily dosing was timed to occur at either CT6 (Circadian Time 6) or ZT11 (Zeitgeber Time 11). Prior to the dose, animals were switched from the prevailing 12:12 LD cycle to a period of constant darkness beginning at either the time of lights-on (ZT0, or 6 h before the "subjective" midday treatment at CT6) or switched at the time of lights-off (ZT12 or shortly after the ZT11 treatment during LD). This method was employed to minimize light cues and thus allow the expression of endogenous rhythms. Animals were kept in constant darkness for approximately 10 days following acute drug administration; 14 days of constant darkness were allowed after implantation of osmotic minipumps.

Dosing of escitalopram at CT6 was chosen as earlier studies had shown this time to be sensitive to serotonergic modulation [14,45]; dosing at ZT11 was chosen to mimic the early morning dose commonly used by patients and to be less disruptive to the normal sleep–wake cycle of the rodents.

2.3. Tissue preparation

Tissue explants containing the SCN from PER2::LUC knock-in mice were prepared according to published methods [43]. Briefly, animals were anesthetized with isoflurane, brains were rapidly removed and 300 μm coronal sections through the SCN were cut on a vibratome (Campden Instruments, Lafayette, IN) in chilled Hank's balanced salt solution (Invitrogen, Carlsbad, CA). Sections were trimmed by hand to include the SCN and a minimum of surrounding tissue and immediately transferred to culture inserts (Millipore, Billerica, MA) in 35 mm dishes (BD Falcon, Franklin Lakes, NJ). Dishes contained 1.2 ml Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO) supplemented with 1× B27 (Invitrogen), 4 mM L-glutamine (Invitrogen), 25 mM glucose (Sigma), 4.2 mM $NaHCO_3$ (Sigma), 10 mM HEPES (Sigma), 25 units/ml penicillin-G sodium (Invitrogen), 34 μM streptomycin sulfate (Invitrogen), and 100 μM beetle luciferin (Promega, Madison, WI, USA) and were sealed with vacuum grease (Dow, Midland, MI) and glass coverslip. Two SCN tissue sections were collected from a single animal; multiple samples of the same tissue from a single animal were placed in different treatment groups.

2.4. Bioluminescence monitoring

SCN tissue cultures were maintained at 35.8 °C, and integrated bioluminescence was collected for 60 s every 10 min with a commercial luminometer (LumiCycle, Actimetrics, Wilmette, IL) before and after treatments. Bioluminescence analysis was performed using LumiCycle Analysis software. Data from the dissection day (Day 0) and from the days with media changes or day 1 of treatment were excluded from the analysis. Bioluminescence traces were de-trended by baseline subtraction of a 24-hour moving average and then smoothed with a 2-hour running average. De-trended and smoothed data were analyzed for periodicity with a $\chi 2$ periodogram (Lumicycle Analysis software; Actimetrics Inc., Evanston, IL). Chi-square tests for the period of Per2-luc expression were considered significant at $p<0.001$. The period of the PER2::LUC expression rhythms was determined with a sine-fit curve [30]; mean values obtained in the control (vehicle) groups ranged from 24.94 ± 0.10 to 25.15 ± 0.14 (Figs. 1 and 2), in keeping with numerous published reports (e.g. [19,21,28]). A minimum of 3 days of bioluminescent data was included in the analysis. Period values were compared with one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparison test. All comparisons were considered significant at $p<0.05$.

2.5. Locomotor activity rhythm monitoring

Daily wheel-running activity of male Sprague–Dawley rats was monitored with Running Wheel Activity Software (AWM V12.0, Lafayette