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Agomelatine affects rat suprachiasmatic nucleus neurons *via* melatonin and serotonin receptors

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ABSTRACT

Aims: The hypothalamic suprachiasmatic nucleus (SCN), which functions as a circadian pacemaker in mammals, is influenced by melatonin and serotonin. Agomelatine, which acts as an antidepressant and can synchronize disturbed circadian rhythms, displays a unique mechanism of action involving both melatonergic agonist and 5-HT_{2C} antagonist properties. This study investigated the dose-dependent effects of agomelatine, melatonin and a selective 5-HT_{2C} receptor antagonist, S32006, on SCN neurons in an *in vitro* slice preparation.
Main methods: Brain slices containing the SCN were prepared from male Wistar rats and maintained in a recording chamber. Changes in firing rates of SCN neurons were recorded after perfusion of drugs.
Key findings: SCN firing rates were dose-dependently suppressed by 19.2–80.9% following perfusion of 0.04–0.32 mM agomelatine ($p < 0.001$, $IC_{50} = 0.14$ mM). Perfusion with melatonin (0.4–3.2 mM) resulted in 16.6–62.5% dose-dependent reductions in firing rates (at least $p < 0.01$, $IC_{50} = 1.59$ mM) and of the duration of suppression. A selective melatonin receptor antagonist (S22153 at 0.32 mM) and a 5-HT_{2c} receptor agonist (Ro60-0175) reduced the suppressive effects of 0.16 mM agomelatine by 35% and 50.2%, respectively. A 5-HT_{2C} receptor antagonist (S32006; 0.03–0.12 mM) significantly decreased SCN firing rates (19.6–91.8%; at least $p < 0.05$, $IC_{50} = 0.05$ mM). Co-perfusion of S32006 (0.06 mM) with a 5-HT_{2C} agonist (Ro60-0175; 0.003 mM) reduced suppressions evoked by S32006 alone by ~72.1%.
Significance: These results are consistent with the hypothesis that agomelatine acts directly on the SCN *via* both agonist effects at melatonergic receptors and antagonist effects at 5-HT_{2C} receptors, which parallel its mechanisms of action as an antidepressant.

1. Introduction

Most currently available drugs used to treat depression act by affecting serotonergic (5-hydroxytryptamine, 5-HT) and/or noradrenergic neurotransmission. A recently developed drug that was approved in 2009 in Europe for the treatment of major depression (agomelatine, S20098; Valdoxan®, Thymanax®) has a unique pharmacological profile [1,2]. It acts both as an agonist at melatonin MT_1 and MT_2 receptors [3–5] and as an antagonist at 5-HT_{2C} receptors [6]. Agomelatine has been shown to have antidepressant and anxiolytic properties in a broad range of animal models and to alleviate symptoms of depression in depressed patients [1,7–11]. Meta-analyses have shown agomelatine to be as effective as traditional antidepressants and to have the benefit of fewer side effects that may lead to discontinuation of treatment [12–15].

There is evidence supporting the idea that the beneficial effects of agomelatine on depression reflect synergy between melatonergic agonist and 5-HT_{2C} antagonist properties [2,7,10,16,17]. This hypothesis is supported by evidence that 5-HT_{2C} and melatonergic receptors are located in a number of brain regions that have been implicated in the pathophysiology of depression, as well as in the suprachiasmatic nuclei (SCN) [18–23], and that the expression of melatonin receptors is changed in depressed patients [24]. The SCN may play a role in the pathophysiology of depression by affecting the regulation of daily rhythms, including the sleep-wake cycle. Sleep disruption is a core symptom of depression seen in up to 80% of patients [25–27], and treatment of sleep problems has been reported to improve the effectiveness of antidepressant treatments [28,29].

Several findings link the SCN specifically to depression. There were reduced numbers of SCN cells immunoreactive for nitric oxide synthase in an animal model of depression [30]. Circadian rhythm disruption

Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; ACSF, artificial cerebrospinal fluid; cAMP, cyclic adenosine monophosphate; DMSO, dimethyl sulfoxide; PLC, phospholipase C; MT, melatonin; SCN, suprachiasmatic nucleus.

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resulting from knockdown of a clock gene selectively in the SCN was associated with helplessness, behavioral despair, and anxiety-like behavior in mice [31] and SCN ablation reduced the anxiolytic effects of agomelatine in an animal model of social defeat [11]. In a *post mortem* study of the brains of people with depression, there were an increased number of melatonin MT_1 receptors and of some peptide-immunoreactive neurons [24]. One route by which the SCN could affect mood regulation is through its indirect outputs to dopaminergic and noradrenergic neurons in the ventral tegmental area and locus coeruleus [32,33].

SCN firing rates are affected by a number of serotonin and melatonin receptor agonists [4,34]. A role specifically for 5-HT_{2C} receptors in SCN function was indicated both by their presence in this nucleus [35] and by evidence that their activation induced changes in expression of the core clock gene *per1* and of the immediate-early gene *c-fos* at some circadian phases [36]. 5-HT_{2C} receptor agonists have been shown to phase shift SCN-regulated rhythms, including that of melatonin production, while a specific 5-HT_{2C} receptor antagonist blocked these phase-shifting effects [37,38].

We showed previously [4] that both agomelatine and melatonin cause dose-dependent suppression of firing rates of most SCN cells studied in Syrian hamsters *in vivo*. The current study was designed to further investigate the roles of melatonin receptors and 5-HT_{2C} receptors in affecting SCN neuronal activity. We used *in vitro* rat SCN slice preparations to study the dose-dependent effects of agomelatine, melatonin and a selective 5-HT_{2C} receptor antagonist, S32006 (N-pyridin-3-yl-1,2-dihydro-3H-benzo[e]indole-3-carboxamide) [39] on rat SCN cell firing rates. We assessed whether a selective melatonin receptor antagonist (S22153) [40] or a highly selective 5-HT_{2C} agonist (Ro60-0175) [41] modified the effects of agomelatine on SCN cell firing rates, and we examined the interactions between Ro60-0175 and the 5-HT_{2C} antagonist S32006.

2. Materials and methods

2.1. Animals and experimental procedures

All experiments were conducted using adult male Wistar rats (90–110 g, 3–4 weeks of age). Animals were obtained from the Laboratory Animal Center of Jilin University (Changchun, China; Certification No. SCXK(Ji)2007-0003) and were maintained in a standard lighting cycle with 12 h light daily (lights on at 07:00), with free access to food and water. All animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals and were approved by the Animal Care and Ethics Committee of Jilin University. All studies complied with the ARRIVE guidelines (Animals in Research: Reporting *In Vivo* Experiments) [42]. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Hypothalamic slice preparation

Animals were anesthetized by injection of urethane (20% solution). When the animal was deeply anesthetized, it was decapitated and the brain was dissected out and transferred immediately to ice-cold artificial cerebrospinal fluid (ACSF) with 95% O_2 and 5% CO_2 for preparation of brain slices. The tissue was trimmed to the hypothalamic region, and coronal brain slices ~350 μm thick containing the SCN were prepared using a vibratome (5000 mz-2, Campden Instruments Ltd, UK). Slices were incubated in ACSF at 34 °C for 25 min and were then allowed to equilibrate in the ACSF at 22–25 °C for at least 1 h before recordings began. Slices were then transferred to the recording chamber and perfused with oxygenated ACSF (22–25 °C), with 95% O_2 and 5% CO_2 at a flow rate of 2 mL/min.

2.3. SCN firing rate recording and drug administration

Experiments were conducted between 15:00 and 22:00 with most recordings made before 20:00; *i.e.*, principally in the late light phase of the colony lighting cycle. Previous studies have found that bath-applied melatonin inhibits rat SCN neurons in slice preparations independently of circadian phase [43–45]. Firing rates of single, discriminated action potentials of ventral SCN neurons were recorded using a glass microelectrode filled with a 2% solution of pontamine sky blue in 0.5 mol/L sodium acetate buffer. Electrode tips were readily visible under magnification, and were guided under visual control into the ventral SCN; cell locations were further confirmed in some slices by ejecting pontamine after completion of a study. Electrode impedance ranged from 9.5 to 13 MΩ. Extracellular potentials were amplified and filtered with a microelectrode amplifier (FWD-1A, Liuhe Radio Factory, Nanjing, China). Action potentials were monitored continuously using a dual-beam storage oscilloscope (VC-10, Nihon Kohden, Tokyo, Japan) and collected and digitized using a data acquisition system at a sampling rate of 10 KHz (PowerLab 4/25; ADI, Sydney, Australia).

To assess nonspecific effects of the dimethyl sulfoxide (DMSO) vehicle, in a subset of studies the vehicle was first perfused onto the slice before applying a target drug. Drugs were obtained from Servier, France (S32006, S20098 [agomelatine] and S22153), Sigma, USA (melatonin) and TOCRIS, UK (Ro60-0175). At all concentrations tested, they were dissolved in 0.01–0.1% DMSO and were then perfused into the slice chamber for 10 min once a baseline firing rate had been established.

2.4. Experimental protocols

2.4.1. Effects of drugs on SCN neuronal firing rates

We investigated the effects of agomelatine, melatonin and S32006 at several concentrations on SCN neuronal firing rates in hypothalamic slice preparations maintained *in vitro*. Drug concentrations (in mM) used in this experiment were as follows: 0.02, 0.04, 0.08, 0.16 and 0.32 for agomelatine; 0.2, 0.4, 0.8, 1.6 and 3.2 for melatonin; 0.015, 0.03, 0.06 and 0.12 for S32006. The drug concentrations used were determined empirically based on identifying the highest concentrations that produced no firing-rate effects under these recording conditions and ranging upwards from that value. The relatively high concentrations of melatonin required may be related to the temperature of the recording chamber or to the degree of penetration of the perfusate into the tissue slices.

2.4.2. Modulation by S22153 or Ro60-0175 of the effects of agomelatine

We first determined that the melatonin receptor antagonist S22153 did not have significant effects on SCN neuronal firing rates over the range of concentrations tested. We then examined its ability to modify the effects of agomelatine on SCN firing rates. For this study, the following approach was used. First, we examined any potential effects of the DMSO vehicle on the firing rates of a single, discriminated SCN neuron and then assessed the effects of agomelatine on these neurons. In another sample of SCN neurons, we first perfused the slice with S22153 (0.32 mM) alone for 10 min, followed by a 10 min perfusion with agomelatine (0.16 mM) combined with S22153 at the same concentration (0.32 mM). The same approach was used to examine how perfusion of Ro60-0175 (0.003 mM) affected suppression of SCN firing rates by agomelatine.

2.4.3. Modulation by Ro60-0175 of the effects of S32006

We first determined whether the 5-HT_{2C} agonist Ro60-0175 affected SCN neuronal firing rates using a range of concentrations, but observed no significant effects. We then examined whether Ro60-0175 altered the effects of the 5-HT_{2C} receptor antagonist, S32006, on SCN cell firing rates. For this study, the following approach was used. First, we examined any potential effects of the DMSO vehicle on the firing rates