Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 527 (2005) 86–93
www.elsevier.com/locate/ejphar

The selective tachykinin neurokinin 1 (NK_1) receptor antagonist, GR 205,171, stereospecifically inhibits light-induced phase advances of hamster circadian activity rhythms

Robert L. Gannon [a,*], Mark J. Millan [b]

[a] Department of Biology, Valdosta State University, Valdosta, GA 31698, USA
[b] Institut de Recherches Servier, Psychopharmacology Department, 125, chemin de ronde, 78290 Croissy-sur-Seine (Paris), France

Received 18 July 2005; received in revised form 27 September 2005; accepted 5 October 2005

Abstract

Circadian rhythms in mammals are generated by master pacemaker cells located within the suprachiasmatic nucleus of the hypothalamus. In hamsters, the suprachiasmatic nucleus contains a small collection of cells immunoreactive for substance P, the endogenous ligand of tachykinin neurokinin 1 (NK_1) receptors. In addition, two other nuclei which form part of the circadian system, the intergeniculate leaflet of the thalamus and the raphe nuclei, also contain fibers and/or cell bodies immunoreactive for substance P. In light of these observations, we evaluated the influence of the selective tachykinin NK_1 receptor antagonist, GR 205,171, upon circadian activity rhythms in the hamster. Systemic injection of GR 205,171 dose-dependently (2.5–40.0 mg/kg, i.p.) inhibited light-induced phase advances in hamster circadian wheel running activity rhythms by approximately 50%. In contrast, GR 226,206, the less active enantiomer of GR 205,171, failed to affect light-induced phase advances. In addition, we examined the potential ability of GR 205,171 to induce non-photic phase shifts in hamster wheel running rhythms when injected at mid-day to late night circadian times. However, GR 205,171 (40 mg/kg) did not elicit non-photic phase shifts at these times indicating that tachykinin NK_1 receptor antagonists are only effective when a light stimulus is applied to the pacemaker. Although GR 205,171 may, in theory, activate several sites within the circadian system, we suggest that GR 205,171 acts in the raphe nuclei to increase inhibitory serotonergic input to pacemaker cells in the suprachiasmatic nuclei, thereby suppressing photic modulation of the pacemaker. These findings have important implications for the use of tachykinin NK_1 receptor antagonists in the treatment of depression and other central nervous system disorders.

Keywords: Circadian; Suprachiasmatic; Neurokinin; Raphe; Depression

1. Introduction

The primary pacemaker for circadian rhythms in mammals is located in the suprachiasmatic nuclei at the base of the hypothalamus (Ralph et al., 1990). The suprachiasmatic nuclei are innervated by retinal axons arriving via the retinohypothalamic tract, and information regarding the light/dark cycle conveyed by this pathway is used by the pacemaker to adjust the timing of the clock to changes in day-length during the year. Glutamate is the primary transmitter of the retinal afferents to the suprachiasmatic nuclei (Ebling, 1996), though pituitary adenylate cyclase activating peptide is well-established to be co-localized with glutamate in retinohypothalamic tract terminals (Chen et al., 1999; Gillette and Mitchell, 2002).

As regards other neuropeptides, there is considerable interest in the potential significance of substance P, the endogenous ligand of tachykinin NK_1 receptors. In fact, there is some disagreement as to whether substance P is also contained within the retinohypothalamic tract terminals, and this appears to differ in a species-dependent fashion. Thus, several reports suggest that substance P is found in the retinohypothalamic tract of rats (Mikkelsen and Larsen, 1993; Piggins et al., 2001), whereas others suggest that it is *not* (Hannibal and Fahrenkrug, 2002; Hartwich et al., 1994; Otori et al., 1993). There is also a report suggesting that substance P is contained within the human retinohypothalamic tract (Moore and Speh, 1994). However, there is a consensus that substance P is *not* part of the

* Corresponding author. Tel.: +1 229 333 5761; fax: +1 229 245 6585.
E-mail address: rlgannon@valdosta.edu (R.L. Gannon).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.10.012

retinohypothalamic tract in hamsters or mice (Hartwich et al., 1994; Piggins et al., 2001).

The suprachiasmatic nuclei also receive information from the intergeniculate leaflet of the thalamus via the geniculohypothalamic tract which contains γ-aminobutyric acid (GABA) and neuropeptide Y as the primary transmitters, while enkephalin is also found in the hamster (Harrington et al., 1985; Morin et al., 1992). The intergeniculate leaflets of rat, hamster and mice all contain axons immunoreactive to substance P and tachykinin NK_1 receptors are also localized in this structure (Morin et al., 1992; Piggins et al., 2001). In addition to the intergeniculate leaflet, the suprachiasmatic nuclei are innervated by brainstem raphe nuclei, in particular the median raphe in hamsters (Meyer-Bernstein and Morin, 1996). Although raphe input to the suprachiasmatic nuclei is primarily serotonergic, substance P and tachykinin NK_1 receptors are found within the raphe nuclei (Leger et al., 2002; Meyer-Bernstein and Morin, 1996), and are believed to modulate the output of these nuclei (see Discussion). Finally, there is a small group of substance P immunoreactive cells located within the central region of the suprachiasmatic nuclei in the hamster, rat and primate, but not the mouse (Hartwich et al., 1994; Mick et al., 1992; Mikkelsen and Larsen, 1993; Morin et al., 1992; Piggins et al., 2001; Reuss et al., 1994). Therefore, in the hamster, substance P could influence circadian rhythmicity by acting in the suprachiasmatic nuclei, intergeniculate leaflet, or raphe nuclei.

The potential influence of substance P upon circadian rhythms has been investigated in both rats and hamsters. In the rat hypothalamic slice in vitro, substance P shifts the rhythm of spontaneous neuronal firing in a manner analogous to that of light (Shibata et al., 1992). Also in the slice, optic nerve-evoked phase shifts in suprachiasmatic nuclei neuronal firing are inhibited by tachykinin NK_1 receptor antagonists while exogenously applied substance P elicits shifts in neuronal firing frequencies (Hamada et al., 1999; Kim et al., 2001). The phase-shifting effects of substance P are thought to result from an increase in glutamate release from the rat retinohypothalamic tract (Hamada et al., 1999; Kim et al., 1999, 2001). In the hamster, tachykinin NK_1 receptor antagonists block light-induced c-*fos* expression in the suprachiasmatic nuclei (Abe et al., 1996), as well as phase advances in circadian activity rhythms (Challet et al., 1998, 2001), following i.c.v. or systemic injections, respectively. Thus, the site of action for tachykinin NK_1 receptor antagonists in the hamster has not been definitively linked to the suprachiasmatic nuclei, but may be acting elsewhere in the circadian system. However, in both the rat and hamster, substance P mimics the effect of light on the circadian pacemaker.

Pharmacological studies of circadian rhythms are important inasmuch as novel drugs are needed for the normalization of dysfunctional circadian rhythms in central nervous system disorders such as Alzheimer's and Parkinson's disease, insomnia and depression (Harper et al., 2001; Reid et al., 2004). Moreover, irregularities in human circadian rhythms have been specifically implicated, but not unequivocally confirmed, in the aetiology of Seasonal Affective Disorder (Burgess et al., 2004; Koorengevel et al., 2003; Magnusson and Boivin, 2003; Rosenthal et al., 1984). As regards potential therapeutic mechanisms, an evaluation of the influence of tachykinin NK_1 receptor antagonists upon circadian rhythms is of special interest in view of their current development for the treatment of major depression and anxiety disorders (Duffy, 2004; Millan, 2003; Rupniak and Kramer, 1999; Rupniak et al., 2001).

In the present study we examined the ability of the highly selective tachykinin NK_1 receptor antagonist GR 205,171 to modulate both light-induced and non-photic phase shifts in hamster circadian activity rhythms. GR 205,171 is the most selective and best characterized tachykinin NK_1 receptor antagonist designed to date (Gardner et al., 1996; Millan et al., 2001; Saria, 1999). To confirm the specificity of its potential actions, we examined in parallel the actions of its less active stereoiosmer, GR 206,226. In addition, potential non-photic activity of tachykinin NK_1 receptor antagonists acting at times other than late in the mid-day is reported here for the first time. Finally, recent evidence from other laboratories allows us to propose a truly novel site and mechanism of action for the phase-modulating activity of tachykinin NK_1 receptor antagonists, the raphe nuclei.

2. Materials and methods

Young (80 g) male Syrian hamsters (*Mesocricetus auratus*) were purchased from Charles River Laboratories (Kingston, NY) and maintained in a 14 : 10 h light : dark cycle for at least two weeks prior to use in experiments. Food and water were provided ad libitum. Hamsters were transferred to individual cages equipped with small running wheels (19 cm diameter) and placed in conditions of constant darkness for the duration of each experiment (about three weeks in constant darkness). Wheel revolutions were identified using magnets and magnetic switches attached to the running wheel and cage lid, respectively, and recorded using hardware and software from Actimetrics (Wilmette, IL, USA) and Matlab (The MathWorks, Natick, MA, USA). All experiments were approved by the Institutional Animal Care and Use Committee and conform to the European Community guidelines for the use of experimental animals.

The time of wheel running onset by hamsters in constant darkness each day is identified as circadian time 12 (12 h of a 24 h day). For each experiment day, circadian time 12 was determined by fitting a line through circadian time 12 of the previous five days, and extrapolating for circadian time 12 the next day. Phase shifts in activity onsets were determined by fitting a line through days five–ten post-experiment, and then comparing the intercept difference between the slopes of the pre- and post-experiment lines on the day of the experiment.

For photic experiments, after the hamsters had been in constant darkness for approximately ten days, the hamsters were removed from their cages in constant darkness under dim red light (<1 lx), weighed, and injected with either drug or vehicle and returned to their home cage in constant darkness. Forty-five minutes later at circadian time 19, hamsters were