Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1046 (2005) 105 – 115
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

MDMA alters the response of the mammalian circadian clock in hamsters: Effects on re-entrainment and triazolam-induced phase shifts

M. Gardani*, R.N. Blance, S.M. Biello

Department of Psychology, University of Glasgow, 58 Hillhead Street, Glasgow, G12 8QB, UK

Accepted 29 March 2005
Available online 17 May 2005

Abstract

Serotonin (5-hydroxytryptamine or 5-HT) is a neurotransmitter that is involved in a wide range of behavioural and physiological processes. Previous work has indicated that serotonin is important in the regulation of the circadian clock, which is located in the suprachiasmatic nuclei (SCN) of the hypothalamus. 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy'), which is widely used as a recreational drug of abuse, is a serotonin neurotoxin in animals and non-human primates. Previous work has shown that MDMA exposure can alter circadian clock function both in vitro and in vivo.

Evidence shows that 5-HT may have a modulatory role in the regulation of the circadian clock by non-photic stimuli, such as the benzodiazepine triazolam (TRZ). Triazolam is a short-acting benzodiazepine that results in phase advances of the wheel running activity in hamsters when administered during the mid-subjective day. In the present study, male Syrian hamsters treated with TRZ (5 mg/kg) at ZT6 significantly phase advanced their clock. Treatment with MDMA significantly diminished the TRZ induced phase shift in hamsters.

Previous evidence shows the involvement of 5-HT in the re-synchronisation of the endogenous clock to a new shifted light–dark cycle. Untreated animals were successfully entrained to a new, 6 h advanced light–dark cycle within an average of 4.5 ± 0.1 days. Following treatment with MDMA, these animals took an average of 8.3 ± 0.1 days to re-entrain to a shifted environmental cycle.

Immunohistochemical analysis revealed that animals treated with MDMA showed reduced serotonin staining, as evidenced by a decrease in innervation density in the SCN. No significant differences were found in cell counts within the raphe nuclei.

These results demonstrate the importance of the serotonergic system in the modulation of photic and non-photic responses of the circadian pacemaker.

Theme: Neural basis of behaviour
Topic: Biological rhythms and sleep

Keywords: Circadian; Serotonin; 3,4-methylenedioxymethamphetamine; Triazolam; Entrainment

1. Introduction

The hypothalamic suprachiasmatic nuclei (SCN) have been identified as the primary site of the mammalian pacemaker [57]. The SCN receive a direct retinal projection, the retinohypothalamic tract. In addition, there is an indirect visual projection the geniculohypothalamic tract (GHT) originating from the intergeniculate leaflet (IGL) of the lateral geniculate nuclei (LGN) [21]. Finally, a dense serotonergic projection innervates the SCN from the raphe nuclei, which comprises of a direct projection from the midbrain raphe nuclei (MnR) and an indirect projection from the dorsal raphe nuclei (DR) via the IGL [27]. Recent findings reveal a reciprocal connection of the MnR to the DR that is partially serotonergic [20,39].

While light is the principal natural temporal stimulus, the mammalian pacemaker is also responsive to a variety of non-photic stimuli [42]. These include a range of behav-

* Corresponding author. Fax: +44 141 3304606.
E-mail address: maria@psy.gla.ac.uk (M. Gardani).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.03.056

iourally arousing stimuli, such as controlled feeding patterns or exercise [32], novelty-induced wheel running [47,61] and social interactions [41]. The phase response curves for these stimuli are characterised by large phase advances during the subjective day (the rest phase in nocturnal rodents) and smaller (more variable) phase delays during the subjective night [5,64].

Serotonin is a neurotransmitter that is involved in a wide range of behavioural and physiological processes, such as feeding patterns, sex, aggressive behaviour, endocrine regulation, motor activity, pain modulation, learning, memory and mood [23]. It was one of the first neurotransmitters to be associated with the regulation of circadian rhythmicity [24,54] and the SCN has been shown to have a denser serotonergic innervation than anywhere else in the forebrain [36]. Numerous studies have demonstrated that 5-HT can modify the response of the mammalian clock to light. Phase advances to light of the hamster circadian clock can be blocked by electrical stimulation of the MnR [58] that has been reported to increase 5-HT release in the hamster SCN [13]. Serotonin agonists can attenuate or completely inhibit light induced phase shifts as well as *c-fos* expression in the SCN [19,29,53,59,80] and serotonergic antagonists can potentiate both light-induced advances and delays in the hamster circadian system [60,70]. Serotonergic agonists show similar phase resetting effects during the subjective day in intact hamsters [9,72] and in vitro in rat SCN slice preparation [55,56]. The similarities of the phase response curve seen in response to 5-HT or 5-HT agonists with that of other non-photic stimuli suggest a possible role of 5-HT as a mediator of these stimuli.

The short-acting benzodiazepine triazolam (TRZ), which has been widely prescribed for the treatment of insomnia, has been shown to phase shift the circadian clock in a non-photic pattern [44,73,74,76]. Electrical stimulation of the IGL/GHT in hamsters can induce phase shifts similar to those induced by TRZ [65], whereas lesions of the IGL/GHT can block the phase shifting effects of TRZ and other benzodiazepines [4,22]. Depletion of serotonergic neurons in the MnR attenuates significantly the phase resetting effects of TRZ on the circadian clock [28].

Triazolam-induced phase advances are attenuated by depletion with reserpine, a non-selective presynaptic monoamine depletor [51] and by a decrease of 5-HT following administration of the serotonin neurotoxin, p-chloroamphetamine (PCPA) [52]. Selective destruction of serotonergic afferents to the SCN by hypothalamic application of 5,7-dihydroxytryptamine (5,7-DHT) can block the resetting effects of TRZ in hamsters [10]. Meyer-Bernstein and Morin have shown that injections of the 5,7-DHT serotonin neurotoxin in the MnR can block the phase advancing effects of TRZ, while depletion of serotonergic neurons in the DR had no effect [28]. More recently, it has been shown that specific destruction of 5-HT fibres connecting the DR to the IGL leads to a suppression of TRZ-induced phase shifting effects on the hamster circadian pacemaker [66].

A number of non-photic stimuli have been shown to alter the rates of re-entrainment to an advanced light–dark cycle (LD). Confinement to a novel wheel or injections of the short-acting benzodiazepine TRZ can accelerate the resynchronisation to a shifted LD cycle in hamsters [45,75]. Systemic administration of the 5-HT $_{1A/7}$ agonist (±) 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrobromide (8-OH-DPAT) accelerated the rate of re-entrainment to an advanced light–dark cycle in hamsters [72]. In humans, evidence shows that exercise can induce phase shifts on the circadian clock [78] and may also promote re-entrainment [15].

Since both TRZ induced phase advances and re-entrainment to an advanced LD cycle may require the involvement of 5-HT, it is possible that damage which alters the levels of 5-HT may impact on the ability of these stimuli to alter the circadian clock function. The synthetic amphetamine-derivative MDMA is an indirect serotonergic agonist, which is widely used as a recreational drug of abuse [49]. A number of laboratories have demonstrated that brain serotonergic neurotoxicity can result from repeated exposure to MDMA in both rodents and non-human primates [2,48,49,63]. Immunohistochemical techniques have demonstrated that MDMA administration results in loss of fine serotonergic axons throughout the rat forebrain [48]. Behavioural findings show that repeated exposure to MDMA significantly alters the response of the hamster circadian clock to both light and the 5-HT $_{1A/7}$ receptor agonist 8-OH-DPAT in vivo [8]. In addition, in vitro evidence indicates that pre-treatment with MDMA may alter the ability of a 5-HT agonist to phase shift the circadian clock at the level of the SCN [3]. As repeated exposure to MDMA results in serotonergic neurotoxicity, the present study examines the implications of this depletion in the established phase shifting effects of the benzodiazepine TRZ on the hamster circadian clock. Additionally, we investigate whether or not MDMA serotonergic destruction alters the endogenous ability of the mammalian pacemaker to resynchronise to a LD cycle advanced by 6 h. Further, using immunohistochemical techniques, we investigate the effects of MDMA treatment on 5-HT cells and fibers within the SCN and the raphe formation.

2. Materials and methods

2.1. Animals

Experimental procedures were carried out under license by the Home Office (UK) in compliance with the Animals (Scientific Procedures) Act of 1986. Male Syrian hamsters (90 to 100 g, Harlan Sprague–Dawley, Oxon, UK) were housed in individual polypropylene cages (41.5 × 25 × 10.5 cm) with nest boxes (13 × 9 × 8 cm) and fitted with a stainless steel running wheel (16 cm in diameter). Food and water were available ad libitum. The room temperature was monitored and kept constant at 22 ± 2 °C. Upon arrival the