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Research report

The effects of systemic and local microinjection into the central nervous system of the selective serotonin 5-HT6 receptor agonist WAY-208466 on sleep and wakefulness in the rat

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HIGHLIGHTS

- Systemic administration of WAY-208466 increases wakefulness and reduces sleep.
- Microinjection of WAY-208466 into CNS structures specifically suppresses REM sleep.
- WAY-208466 could serve as a biological marker of the therapeutic effects of SSRIs.

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ABSTRACT

The effects of WAY-208466, a selective 5-HT6 receptor agonist on spontaneous sleep were studied in adult rats implanted for chronic sleep recordings. Systemic administration of WAY-208466 during the light phase of the light–dark cycle significantly increased wakefulness (W) and reduced slow wave sleep (SWS), REM sleep (REMS) and the number of REMS periods. Pretreatment with the selective 5-HT6 receptor antagonist RO-399885 prevented the effects of the 5-HT6 receptor agonist on W, SWS and REMS. Direct infusion of WAY-208466 into the dorsal raphe nucleus, locus coeruleus, basal forebrain (horizontal limb of the diagonal band of Broca) or laterodorsal tegmental nucleus specifically decreased REMS without significantly altering W or SWS. In all instances the REMS suppression was dependent upon the reduction of REMS periods. The finding that WAY-208466 increases extracellular γ-aminobutyric acid (GABA) levels in the rat frontal cortex tends to suggest that the neurotransmitter could be involved in the 5-HT6 receptor agonist-induced disruption of the sleep–wake cycle. However, further studies are needed to resolve this issue.

1. Introduction

Within the central nervous system serotonin (5-HT) participates in a great number of functions including sleep–wake behavior, affect, cognition, sexual function, thermoregulation and food intake. It has been established, in addition, that increases or decreases of 5-HT at central sites correlate with improved or worsened depression.

The 5-HT receptors are presently classified into seven classes, designated 5-HT1-7 [12]. The 5-HT6 is a G-protein coupled receptor and its primary signal transduction pathway is the stimulation of adenylate cyclase [29]. The 5-HT6 receptor is located postsynaptically to 5-HT cells and has been localized in the frontal cortex, basal forebrain (bed nucleus of the stria terminalis, diagonal band of Broca, medial septal nucleus), limbic system [hippocampus (CA1, CA2, CA3, CA4 and dentate gyrus), amygdala], striatum, nucleus accumbens, thalamus, hypothalamus (anterior and lateral area) and brainstem (raphe nuclei) of the rat [10,13,15,27,41,42]. The finding that 5-HT6 receptor is located in the frontal cortex, striatum and limbic system, and shows high affinity for atypical antipsychotics, as well as for several antidepressant drugs has led to the proposal that it participates in the control of mood, motivation and motor function, and their disorders [8]. Of note, many terminal regions where 5-HT6 receptor has been localized are involved in the regulation of sleep and W. However, little is known about the potential contribution of 5-HT6 receptor in the regulation of the sleep–wake cycle.

Recently, we reported that systemic administration of the 5-HT6 receptor antagonists SB-399885 and RO-4368554 during the light phase of the light–dark cycle caused a significant increase of W and

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a reduction of slow wave sleep (SWS) and rapid-eye-movement sleep (REMS) in the rat. Injection of SB-399885 during the dark period suppressed also REMS [21].

The present experiments were undertaken to characterize the effects of systemic administration of the selective 5-HT6 receptor agonist WAY-208466 (N-[2-[3-(3-fluorophenylsulfonyl)-1H-pyrolo[2,3-b]pyridin-1-yl]ethyl]-N,N-dimethylamine) on sleep and W, and to determine the potential neural sites that mediate these changes in the rat. To this purpose various doses of the compound were administered intraperitoneally (i.p.) or microinjected into brain regions involved in the regulation of sleep and W, including the dorsal raphe nucleus (DRN), locus coeruleus (LC), lateral basal forebrain [horizontal limb of the diagonal band of Broca (HDB)], and laterodorsal tegmental nucleus (LDT). In order to characterize the selectivity of WAY-208466 for 5-HT6 receptor, its systemic administration in a group of animals was preceded by that of the selective 5-HT6 receptor antagonist SB-399885 {N-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazi- nyl) benzenesulfonamide} at a dose that does not disrupt sleep variables [21].

2. Materials and methods

Thirty four male Wistar rats weighing 300–350 g at the time of surgery were used. All rats were cared for and used in strict accordance with the National Institutes of Health guidelines for the care and use of experimental animals. All procedures were approved by the Institutional Animal Care and Use Committee of the Medical School, Montevideo, Uruguay.

All surgical procedures were performed stereotaxically under aseptic conditions. Sodium pentobarbital (40 mg/kg) was administered i.p. for anesthesia. In addition, the animals were treated postoperatively for four days with the antibiotic cefradine 50 mg/kg, i.m. and the analgesic dipyrone 100 mg/kg, i.m. The rats were implanted with Nichrome® electrodes (200 μm diameter) for chronic sleep recordings of electroencephalogram (EEG) and electromyogram (EMG) activities, through placement on the frontal and occcipital cortices for the former, and on the dorsal neck musculature for the latter. Leads from the recording electrodes were routed to a nine pin miniature plug that mates to one attached to a recording cable. Furacin ointment was applied to the sutured incision. In addition, a guide cannula (26 gauge) was inserted and maintained 2 mm above the: 1) dorsal raphe nucleus (anteroposterior 7.8, lateral 0.0, vertical −5.8); 2) right locus coeruleus nucleus (anteroposterior 9.8, lateral 1.4, vertical −7.0); 3) right and left horizontal limb of the diagonal band of Broca (anteroposterior 0.4, lateral 1.8, vertical −8.6); 4) right laterodorsal tegmental nucleus (anteroposterior 8.7, lateral 0.6, vertical −6.6) [25]. The recording plug and the cannulae were affixed to the skull with dental acrylic and anchor screws. Drug or vehicle was injected into the brain structures with an injection cannula (29 gauge) which extended 2 mm beyond the guide, in a 0.2 μl volume over a 2-min period. On completion of the microinjections, we identified the injection site(s) by the microinjection of Pontamine Sky-blue dye (0.2 μl) into the neural structures. The rats were deeply anesthetized with an overdose of pentobarbital (100 mg/kg, i.p.) and their brains were removed and immersed in paraformaldehyde 4%. Thereafter the brains were cut in 50 μm coronal sections with a vibratome. Selected sections were stained with Pyronin-γ and photographed. Correctness of the cannulae/injection sites was assessed using the atlas of Paxinos and Watson [25]. All the data presented in this report are derived from animals whose injection site was within the limits of the above mentioned brain structures.

The animals were housed individually in a temperature-controlled room (23 ± 1°) under a 12-h light/12-h dark cycle (lights went on at 06:00 h) and with food and water provided ad libitum. Ten days after surgery the animals were habituated to a soundproof chamber fitted with slip-rings and cable connectors, and to the injection procedure. Thereafter, they were administered either a control solution or the drug to be tested. Standard recordings of the EEG and EMG signals were performed. After three consecutive stable recording sessions of 6 h (<10% fluctuation in sleep and W parameters among recordings), experimental microinjections were initiated. Data was collected (Akonic MINI-PC-Windows, Argentine) and an expert scorer who was blind to experimental treatment classified each 10-s epoch as either W [low voltage fast waves in frontal cortex, a mixed theta rhythm (4–7 Hz) in occipital cortex and relatively high electromyographic activity]; light sleep (LS – high voltage slow cortical waves interrupted by low voltage fast electroencephalographic activity); SWS (continuous high amplitude slow frontal and occipital waves combined with a reduced electromyogram); and REMS (low voltage fast frontal waves, a regular theta rhythm in the occipital cortex, and a silent electromyogram except for occasional myoclonic twitching). The time spent in W, LS, SWS and REMS was analyzed into 3 blocks of 2 h duration. Slow wave sleep and REMS latencies, and the number of REM periods were also determined [22].

The doses of WAY-208466 selected for the present study were based on pilot work in our laboratory and the limited previous research in which administration of the compound was employed to determine its pharmacological and neurochemical characteristics [32].

2.1. Experiment 1 (group 1)

WAY-208466 (Pfizer, Groton, CT, USA) 10, 20 and 30 mg/kg or vehicle were administered i.p. ($n = 8$) during the light phase of the 12 h light/12 h dark cycle starting 2 h after the beginning of the light period. The 5-HT6 receptor agonist was dissolved in distilled water which served as vehicle.

2.2. Experiment 2 (group 2)

In the second set of experiments 30 mg/kg WAY-208466 was injected into animals pretreated with 2.5 mg/kg of the selective 5-HT6 receptor antagonist SB-399885 (GlaxoSmithKline, Harlow, UK) dissolved in 1% aqueous solution of Tween 80 ($n = 6$). The drugs were given i.p. 20 min apart in these interaction experiments.

2.3. Experiment 3 (group 3)

WAY-208466 2, 4 or 6 mM (0.69, 1.38 or 2.07 mg/ml) or vehicle (distilled water) was microinjected into the DRN ($n = 6$).

2.4. Experiment 4 (group 4)

WAY-208466 2, 4 and 6 mM or vehicle (distilled water) was microinjected into the right LC ($n = 6$).

2.5. Experiment 5 (group 5)

WAY-208466 1, 2 and 4 mM (0.35, 0.69 or 1.38 mg/ml) or vehicle (distilled water) was microinjected into the right and left HDB ($n = 7$).

2.6. Experiment 6 (group 6)

WAY-208466 1, 2 and 4 mM or vehicle (distilled water) was microinjected into the right LDT ($n = 7$).

Recordings were begun 15 min later and continued for 6 h. The control solution and the active drugs were given at least three days apart.

A repeated measures analysis of variance (ANOVA) using dose as the between subject-factor was performed, with multiple post hoc comparisons carried out with the Dunnett multiple comparisons test when the ANOVA indicated significance ($P < 0.05$).

3. Results

3.1. Effects of systemic administration of WAY-208466

Analysis of sleep variables in 2-h blocks showed that WAY-208466 30 mg/kg i.p. increased W ($F_{(3,21)} = 5.76$, $P < 0.01$) and reduced SWS ($F_{(3,21)} = 3.06$, $P < 0.05$) during the first 2 h of recording. In addition, the 30 mg/kg dose significantly reduced REMS in the first ($F_{(3,21)} = 3.57$, $P < 0.01$) and second ($F_{(3,21)} = 2.64$, $P < 0.05$) 2 h blocks, whereas the 20 mg/kg dose decreased REMS ($F_{(3,21)} = 3.16$, $P < 0.05$) during the first 2-h recording period (Fig. 1). Administration of WAY-208466 30 mg/kg significantly augmented REMS latency ($F_{(3,21)} = 2.76$, $P < 0.05$), while the number of REMS periods showed a significant decrease after injection of the whole range of doses of the 5-HT6 receptor agonist during the first 2 h of recording ($F_{(3,21)} = 2.64$, $P < 0.05$; $F_{(3,21)} = 3.66$, $P < 0.01$; $F_{(3,21)} = 4.27$, $P < 0.01$, respectively), and following the 30 mg/kg dose during the second 2-h period ($F_{(3,21)} = 3.03$, $P < 0.05$) (Table 1).

3.2. Effects of systemic administration of WAY-208466 into animals pretreated with SB-399885

SB-399885 2.5 mg/kg, i.p. prevented the increase of W ($F_{(3,15)} = 2.78$, $P < 0.05$) and the reduction of SWS ($F_{(3,15)} = 2.61$, $P < 0.05$) and REMS ($F_{(3,15)} = 4.16$, $P < 0.01$) induced by WAY-208466 30 mg/kg, i.p. during the first 2-h recording period (Fig. 2). In addition, the compound partly prevented the increase of REMS latency and the reduction of the number of REMS periods during 1–2 h post-injection (Table 2).