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Research report

Systemic administration and local microinjection into the central nervous system of the 5-HT$_7$ receptor agonist LP-211 modify the sleep-wake cycle in the rat

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HIGHLIGHTS

- Systemic administration of LP-211 increases wakefulness and reduces REM sleep.
- Microinjection of LP-211 into the DRN, LC, BFB or LDT suppresses REM sleep.
- Similar changes were described after administration of other 5-HT receptor agonists.

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ABSTRACT

The effects of LP-211, a selective serotonin 5-HT$_7$ receptor agonist were studied in adult rats implanted for chronic sleep recordings. Intraperitoneal administration of LP-211 (2.5–10 mg/kg) during the light phase of the light–dark cycle significantly increased wakefulness (W) and reduced rapid-eye-movement sleep (REMS) and the number of REM periods during the 6-h recording period. Direct infusion of LP-211 into the dorsal raphe nucleus (DRN) (2–6 mM), locus coeruleus nucleus (LC) (4 mM), basal forebrain (horizontal limb of the diagonal band of Broca) (HDB) (2 mM) or laterodorsal tegmental nucleus (LDT) (4 mM) induced also a decrease of REMS. Additionally, microinjection of the 5-HT$_7$ receptor ligand into the HDB (2 mM) augmented W. Presently, there is no satisfactory explanation for the effect of 5-HT$_7$ receptor activation on W and REMS occurrence. Additional studies are required to characterize the neurotransmitter systems responsible for the actions of LP-211 on the behavioral states.

1. Introduction

Within the central nervous system serotonin (5-HT) participates in a great number of functions including sleep–wake behavior, cognition, affect, memory, motivation, sexual function, thermoregulation and food intake [1,17,18].

Serotonin is synthesized in the central nervous system (CNS) by neurons within the raphe nuclei of the brainstem. The two major midbrain nuclei contributing ascending serotonergic innervation are the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN). Serotonergic neurons of the DRN and the MRN innervate brain areas involved in sleep/wake regulation, including the dopaminergic neurons of the ventral tegmental area/substantia nigra pars compacta; the cholinergic nuclei of the mesencephalon and the basal forebrain (BFB); the noradrenergic cells of the locus coeruleus nucleus (LC); the γ-aminobutyric (GABA)-ergic, histaminergic and orexinergic cell aggregates of the hypothalamus, and the glutamatergic neurons of the BFB, thalamus and brainstem reticular formation [19].

Based on electrophysiological, neurochemical, genetic and neuropharmacological approaches, it is currently accepted that 5-HT functions to promote wakefulness (W) and to inhibit rapid-eye-movement sleep (REMS) [18]. In this respect, it has been shown that systemic or intracerebroventricular (icv.) administration of 5-HT1A, 5-HT1B, 5-HT2A/2C, 5-HT2C, 5-HT3, and 5-HT6 receptor agonists significantly increases W and reduces REMS in the rat [18].

Abbreviations: BFB, basal forebrain; CNS, central nervous system; DRN, dorsal raphe nucleus; EEG, electroencephalogram; EMG, electromyogram; 5-HT, serotonin; GABA, γ-aminobutyric acid; HDB, horizontal limb of the diagonal band of Broca; LC, locus coeruleus nucleus; LDT, laterodorsal tegmental nucleus; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei; LS, light sleep; PRF, pontine reticular formation; REMS, rapid-eye-movement sleep; SCN, suprachiasmatic nucleus; SWS, slow wave sleep; W, wakefulness.

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The 5-HT_7 receptor is part of the G-protein superfamily of receptors that contain seven transmembrane regions and its stimulation leads to an increase in cAMP production [33]. Receptor autoradiographic and in situ hybridization analysis indicate the presence of the 5-HT_7 receptor in a number of rat brain areas involved in the regulation of the behavioral states and circadian rhythms, including the cerebral cortex, BFB (medial septal nucleus, diagonal band of Broca, and substantia innominata), hippocampus (fields CA1 and CA3 of Ammon's horn), thalamus (anterior, lateral, medial and posterior nuclei), hypothalamus [suprachiasmatic nucleus (SCN) and tuberomammillary nucleus], and midbrain [laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) and DRN] [9,23,35]. Moreover, immunocytochemistry studies in the mouse have shown the 5-HT_7 receptor to be expressed on GABAergic neurons within the SCN and the DRN [8,27,28,32].

To date, three different approaches have been followed to characterize the role of the 5-HT_7 receptor in the regulation of sleep variables: (1) Hedlund et al. [11] established that 5-HT_7 receptor knockout mice spend less time in REMS during the light phase compared with their wild-type counterparts. On the other hand, there was no difference between the genotypes in time spent in W or slow wave sleep (SWS); (2) Intraperitoneal (i.p.) administration of the potent 5-HT_7 receptor antagonists SB-269970 and SB-656104 to rats at the beginning of the light phase was shown to reduce the total amount of REMS and to increase REMS latency. Values of W and SWS were not significantly modified [10,34]; (3) infusion of SB-269970 or the selective 5-HT_7 receptor agonist LP-44 into the DRN induced also the suppression of REMS in the rat [20,21]. The finding that REMS is reduced by activation as well as blockade of 5-HT_7 receptor led Matthys et al. [16] to propose that "the serotonergic receptor could reside in different dimeric contexts and initiate different signaling pathways, according to the neuronal circuitry and/or brain region involved".

Recently, a series of arylpiperazine derivatives have been developed as potential 5-HT_7 receptor agonists. Among these is LP-211 [N-(4-cyanophenylmethyl)-4-(2-diphenyl)-1-piperazinehexanamide], a brain penetrant compound that undergoes metabolic degradation to 1-(2-diphenyl)piperazine. LP-211 binds with high affinity (K_i = 15 nM) at human cloned 5-HT_7 receptors. Radioligand binding assays have determined that the selectivity of LP-211 against 5-HT1B, 5-HT2B, 5-HT2C and 5-HT5A receptor is discreet (5–14 fold), whereas higher selectivities have been observed for the 5-HT1A, 5-HT1D, 5-HT2A and 5-HT6 receptor subtypes (25–105 fold). In contrast, no significant binding at 5-HT3 and 5-HT1E receptor or at 5-HT transporter was detected [12,13].

The present experiments were undertaken to characterize the effects of systemic administration of LP-211 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 i.p. or microinjected into brain regions involved in the regulation of sleep and W, including the DRN, LC, lateral BFB [horizontal limb of the diagonal band of Broca (HDB)], and laterodorsal tegmental nucleus (LDT).

2. Materials and methods

Thirty male Wistar rats weighing 300–350 g at the time of surgery were used. All rats were cared 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 occipital 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 (27 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 a 1 μl syringe that was associated with an injection cannula (29 gauge) which extended 2 mm beyond the guide, in a 0.2 μl volume over a 2-min period. Although we verified that the cannula tips were confined within the limits of the DRN, LC, HDB and LDT, it is not possible to discard the diffusion of LP-211 outside these neuroanatomical structures. In this respect, it is well-known that the diffusion rate of a given drug depends upon a number of factors including its diffusion coefficient, and the characteristics of the brain region where the microinjection is performed [24]. However, it should be taken into consideration that methylene blue microinjected into the CNS in a 0.2 μl volume diffuses an average ratio of 520 μm [14]. Because of the small doses and volume employed in the present study we consider that the diffusion of effective concentrations of LP-211 outside the DR, LC, HDB and LDT, if present, was negligible. We identified the injection(s) sites three days after completion of the microinjections by the local administration 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 (Figs. 1–4).

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 sound-proof chamber fitted with slip-rings and cable connectors, and to the injection procedure. After three consecutive stable recording sessions of 6 h (<10% fluctuation in sleep and W parameters among recordings), experimental injections 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, and during the 6-h electroencephalographic and electromyographic recordings. Slow