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Research report

Activation of serotonin 5-HT_{1B} receptor in the dorsal raphe nucleus affects REM sleep in the rat

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ABSTRACT

The effects of CP-94253, a selective 5-HT_{1B} receptor agonist, and of SB 224-289, a selective 5-HT_{1B} receptor antagonist, on spontaneous sleep were studied in adult rats implanted for chronic sleep recordings. The 5-HT_{1B} receptor ligands were microinjected directly into the dorsal raphe nucleus (DRN) during the light period of the 12-h light/12-h dark cycle. Infusion of CP-94253 (1–4 mM) into the DRN induced a significant reduction of rapid-eye-movement sleep (REMS) and of the mean duration of REM episodes. On the other hand, SB 224-289 (0.25–0.5 mM) decreased REMS and the number of REM periods. Pretreatment with SB 224-289 (0.125–0.25 mM) antagonized the CP-94253 (4 mM)-induced reduction of REMS and of the mean duration of REM periods. Administration of the $GABA_A$ receptor agonist muscimol (1.5 mM), which by itself did not significantly affect sleep variables, prevented the effect of CP-94253 (4 mM) on REMS suppression. It is proposed that the suppression of REMS after microinjection of CP-94253 into the DRN is related to the inhibition of GABAergic interneurons that make synaptic contacts with serotonergic cells. The resultant increase of serotonin release at postsynaptic sites involved in the induction and maintenance of REMS would induce the suppression of the behavioral state.

1. Introduction

Although many questions remain about the complicated role of serotonin (5-HT) and its receptors in regulating sleep and waking, recent neurochemical, electrophysiological, genetic and neuropharmacological studies have revealed much detailed information about this process. Based on such approaches it is currently accepted that 5-HT functions to promote waking (W) and to inhibit rapid-eye movement sleep (REMS).

The serotonin-containing neurons of the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) provide the principal source of serotonergic innervation of the telencephalon, diencephalon, mesencephalon, and rhombencephalon of laboratory animals and man [28,38]. In this respect, 5-HT neurons of the DRN and the MRN innervate brain areas involved in sleep/wake regulation. These areas include the cholinergic nuclei of the mesencephalon and the basal forebrain, the dopaminergic neurons of the ventral tegmental area and the substantia nigra compacta, the noradrenergic cells of the locus coeruleus, the GABAergic, histaminergic and orexinergic cell aggregates of the hypothalamus and the glutamatergic neurons of the thalamus and the brain stem reticular formation [28].

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) plays an important role in the initiation and maintenance of slow wave sleep (SWS) and REMS. GABAergic neurons that project to the DRN are located in several neuroanatomical structures including the basal forebrain, the hypothalamus, the mesencephalon and the rhombencephalon [24]. In addition, GABAergic interneurons have been described in the DRN [11]. GABA has been made responsible for the reduction of the activity of 5-HT neurons during SWS and REMS. Accordingly, iontophoretic application of GABA decreases the activity of DRN 5-HT neurons and this effect is antagonized by picrotoxin [16]. Furthermore, Nitz and Siegel [31] have found that REMS is accompanied by a significant increase in GABA release in the DRN and that local administration of muscimol into the raphe nucleus increases REMS, whereas picrotoxin blocks its occurrence.

The DRN contains 5-HT and non-5-HT neurons. The latter express a variety of substances including dopamine, GABA and glutamate. In addition, nitric oxide and a variety of neuropeptides have been characterized in the DRN, some of them being coexpressed in 5-HT cells [9,10,23,36].

The serotonergic cells are present throughout the rostral–caudal extent of the DRN, in all clusters of the nucleus. However, they predominate along the midline of the rostral, dorsal and ventral subdivisions of the DRN [11,25]. GABAergic neurons are also abundant throughout the DRN of the rat. Although all DRN subnuclei contain GABAergic interneurons, they predominate in the lateral wings of the raphe nucleus [7,11,15]. A relatively small

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number of glutamatergic neurons have been characterized in the rostral subdivision of the DRN [34]. Moreover, glutamatergic inputs to the DRN have been described that originate in the medial prefrontal cortex, the amygdala, various hypothalamic areas, the parabrachial nuclei, and the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) among others [21].

Allers and Sharp [2] successfully identified the neurochemical and morphological properties of 5-HT-containing cells and GABA-containing cells in the DRN of urethane-anesthetized rats. The slow-firing cells were immunoreactive for 5-HT and/or tryptophan hydroxylase and were distributed throughout the rostral–caudal extent of the DRN. Intravenous administration of the serotonin 5-HT_{1A} receptor agonist 8-OH-DPAT inhibited the activity of the slow-firing cells. The fast-firing neurons were immunoreactive for glutamic acid decarboxylase (GAD), the enzyme that catalyzes the synthesis of GABA, and immunonegative for tryptophan hydroxylase, and were predominantly located in the lateral subdivisions of the DRN.

The 5-HT receptors can be classified into seven classes, designated 5-HT_{1-7}. The 5-HT_1 class consists of five (5-$HT_{1A\text{-}B\text{-}D\text{-}E\text{-}F}$) subtypes [18]. The 5-$HT_{1B}$ receptor is linked to the inhibition of adenylate cyclase and decreased production of cAMP. The activation of 5-HT_{1B} receptor results in membrane hyperpolarization, leading to an inhibition of neuronal firing [30]. The 5-HT_{1B} receptor was initially proposed to be located on 5-HT axon terminals (presynaptic autoreceptor) and postsynaptically (outside the DRN) on non-5-HT neurons. Additionally, a high density of 5-HT_{1B} receptor mRNA has been detected in the raphe nuclei of the rat [6,13,39]. The density of 5-HT_{1B} receptor at the DRN is greatest in the ventromedial cluster, and it is predominantly expressed by non-5-HT cells [8]. The finding that administration of the selective 5-HT_{1B} receptor agonist CP-94253 increases the firing of 5-HT neurons and of dialysate 5-HT in the DRN, seems compatible with the activation of 5-HT_{1B} receptors located in inhibitory, tentatively GABAergic, interneurons [1,14].

The 5-HT_{1B} receptor is involved in the regulation of the synaptic release of 5-HT and of other neurotransmitters including acetylcholine, norepinephrine, dopamine, GABA and glutamate, which is indicative of its role as auto- and heteroreceptor, respectively. In addition, available evidence tends to suggest that the activation of 5-HT_{1B} receptor expressed by inhibitory interneurons that make synaptic contacts with 5-HT cells, indirectly facilitates their functional activity [14].

Few studies has been published on the effect of 5-HT_{1B} receptor ligands on sleep variables. Systemic administration of the 5-HT_{1B} agonists CGS 12066B or CP-94253 significantly increased W and reduced SWS and REMS in the rat [4,27]. The mixed β-adrenoceptor/5-$HT_{1A/1B}$ receptor antagonist pindolol prevented the increase of W and reduction of SWS by CP-94253. However, pindolol failed to prevent the suppression of REMS [27]. Furthermore, quantization of spontaneous sleep–waking cycles in 5-HT_{1B} receptor knockout mice has shown that REMS is increased during the light phase [5].

Recently, Gyongyosi et al. [17] studied the effect of CP-94253 on sleep and W in rats pretreated with 3,4-methylenedioxymethamphetamine (MNMA) six months earlier. MNMA lesioned 5-HT nerve endings in the striatum and prevented the increase of active W observed after systemic administration of the 5-HT_{1B} receptor agonist in the control animals. On the other hand, the CP-94253-induced reduction of SWS and REMS was still present in the MNMA-pretreated rats.

Thus, the limited available evidence tends to indicate that 5-HT_{1B} receptor activation facilitates the occurrence of W and negatively influences SWS and REMS.

To date, no investigations into the role of 5-HT_{1B} receptors present in the DRN in sleep and W have been reported. However, a role for these receptors in the regulation of the behavioral state may be anticipated based on the association of 5-HT neurons with brain regions known to be important in the regulation of W and REMS [28].

The present experiments were undertaken to test the hypothesis that stimulation of the 5-HT_{1B} receptor in the DRN should negatively influence REMS occurrence. For this purpose we made use of the selective 5-HT_{1B} receptor agonist CP-94253 {3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-b]pyridine} [20]. Several doses of CP-94253 were injected into the DRN of animals prepared for chronic sleep recordings. In addition, we tested the potential use of the selective 5-HT_{1B} receptor antagonist SB 224-289 {5,1′-methyl-5-[[2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl]-2,3,67-tetrahydrospiro [furo[2,3-f]indole-3,4′-piperidine]} [35,37] to counteract the CP-94253-induced changes of REMS. We considered also the possibility that the effects induced by CP-94253 are, at least in part, via the inhibition of local GABAergic interneurons. To this purpose we tested the ability of the selective $GABA_A$ receptor agonist muscimol to reverse the CP-94253-induced reduction of REMS.

2. Materials and methods

2.1. Animals

Five different groups of male Wistar rats weighing 320–350 g at the time of surgery were used. All experiments were conducted in accordance with the National Institutes of Health (USA) guidelines for the care and use of laboratory animals. All procedures were approved by the Institutional Animal Care and Use Committee of the Medical School, Montevideo, Uruguay.

2.2. Surgical procedures

All surgical procedures were performed stereotaxically under aseptic conditions. Sodium pentobarbital (40 mg/kg) was administered intraperitoneally for anesthesia. In addition, the animals were treated postoperatively for 4 days with the antibiotic penicillin 50 mg/kg. 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 the 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. A guide cannula (26 gauge) constructed for microinjection into the DRN was implanted with its tip 2 mm above the DRN (AP 7.8; L 0.0; V −5.8) [33]. The recording plug and the cannula were affixed to the skull with dental acrylic and anchor screws. Drug or vehicle was injected into the DRN 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 by the microinjection of Pontamine Sky-blue dye (0.2 μl) into the DRN. The rats were sacrificied with an overdose of pentobarbital, perfused with 4% paraformaldehyde and their brains were removed. Thereafter, the brains were cryoprotected in a solution of sucrose 30%, and cut in 40 μm coronal sections with a cryostat. Selected sections were stained with tionin acetate (Merck, Germany). Correctness of the cannula/injection sites was assessed using the atlas of Paxinos and Watson [33]. All the data presented in this report are derived from animals whose injection site was within the limits of the DRN.

2.3. Recording and sleep scoring

The animals were housed individually in a temperature-controlled room (23 ± 1 °C) under a 12-h light/12-h dark cycle (the 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 procedures. The drugs were always administered during the light phase of the 12-h light/12-h dark cycle, at approximately 08:00 h. A balanced order of drug and control injections was always used to merge the effects of both the drug and the time elapsed during the protocol. Recording was begun 15 min later and continued for 6 h. The predominant activity of each 10 s epoch was assigned to one of the following categories: W, light sleep (LS), SWS, or REMS. Slow wave sleep and REMS latencies, and the number and mean duration of REM periods were also determined [26].

2.4. Experimental design

The doses of CP-94253 (1–4 mM), SB 224-289 (0.125–0.5 mM) and muscimol (1–1.5 mM) selected for the present study were based on pilot work in our laboratory and the limited previous research in which administration of these compounds was