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The effect of $GABA_A$ antagonist bicuculline on dorsal raphe nucleus and frontal cortex extracellular serotonin: A window on SWS and REM sleep modulation

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Abstract

We investigated the effects of the perfusion of γ-aminobutyric $acid_A$ antagonist bicuculline in the dorsal raphe nucleus, on brain 5-hydroxytryptamine level and on sleep. Perfusion of 25 and 50 μM bicuculline into the dorsal raphe nucleus dose-dependently increased dorsal raphe nucleus 5-hydroxytryptamine level during sleep and wakefulness. Frontal cortex 5-hydroxytryptamine level was not affected by either 25 or 50 μM perfusion.

25 μM bicuculline produced only minimal effects on sleep. 50 μM decreased rapid eye movement sleep, slow wave sleep 1 and 2 and increased waking.

Sleep changes leveled out towards the end of the bicuculline perfusion despite serotonin levels were still elevated. This suggests that an adaptation mechanism may take place in order to counteract the high serotonergic output, producing uncoupling between serotonin level and behavioural state. The results support the notion that γ-aminobutyric acid is a strong modulator of dorsal raphe nucleus serotonergic neurons, and that this modulation is important in the regulation of slow wave sleep, rapid eye movement sleep and waking.

Keywords: Sleep–waking; Brainstem; Rat; Microdialysis; HPLC-EC

1. Introduction

The role of the serotonergic system, and most importantly the dorsal raphe nucleus (DRN) in sleep regulation has been debated during the last 40 years (see reviews: Portas et al., 2000; Ursin, 2002).

One line of reasoning favours the possibility that DRN 5-hydroxytryptamine (5-HT, serotonin) neurons are important in maintaining waking (W) and inhibiting rapid eye movement (REM) sleep (serotonergic modulation of REM sleep; McCarley et al., 1995). This is mainly based on 3 lines of evidence: i) serotonergic neurons in the DRN show a state-related firing (higher in W, lower in slow wave sleep (SWS), and lowest in REM sleep) (Fornal et al., 1994; Lydic et al., 1987; McGinty

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; DRN, dorsal raphe nucleus; ECD, electrochemical detection; EDTA, ethylenediaminetetraacetic acid; EEG, electroencephalogram; EMG, electromyogram; FC, frontal cortex; GABA, gamma amino butyric acid; HPLC, high performance liquid chromatography; I.P., intraperitoneally; IPSP, inhibitory postsynaptic potentials; LDT, laterodorsal tegmental nucleus; PAG, periaqueductal grey; PPT, pedunculopontine tegmental nucleus; PRF, pontine reticular formation; REM sleep, rapid eye movement sleep; S.C., subcutaneously; SWS, slow wave sleep; VLPO, ventrolateral preoptic area; W, waking.

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and Harper, 1976; Trulson and Jacobs, 1979), ii) extracellular levels of 5-HT within the DRN (Portas et al., 1996, 1998; Portas and McCarley, 1994) and in the serotonergic projection areas (Auerbach et al., 1989; Horner et al., 1997; Orosco et al., 1995; Strecker et al., 1999; Wilkinson et al., 1991) similarly vary with state, iii) inhibition of REM promoting neurons by 5-HT change according to the DRN neurons firing (W>SWS>REM) (e.g. suppress REM sleep) (Horner et al., 1997). In line with this hypothesis, the modulation of the activity of the DRN serotonergic neurons would directly affect sleep and waking (e.g. high serotonin levels are associated with waking, low serotonin levels are associated with sleep). An autoinhibitory mechanism has been postulated (Wang and Aghajanian, 1978) on the evidence that stimulation of the 5-HT receptors present on the soma (autoreceptors) decreases DRN neuronal firing (e.g. Evrard et al., 1999; Fornal et al., 1994; Sprouse and Aghajanian, 1987) and DRN 5-HT level (Portas et al., 1996). A concomitant increase of REM sleep has consistently been observed (Bjorvatn et al., 1997; Portas et al., 1996) supporting the role of serotonin in the modulation of REM sleep (McCarley et al., 1995). Consistent with these findings systemic administration of the 5-HT_{1A} antagonist p-MPPI dose-dependently decreases REM sleep (Sørensen et al., 2000). However, recent experiments in our laboratory showed that microdialysis perfusion of p-MPPI into DRN produces only minor (Sørensen et al., 2001) or no effects on REM sleep despite a dramatic increase of 5-HT levels. (Ursin et al., unpublished observations).

Another important mechanism suggested for modulation of DRN serotonergic neurons is provided by the γ-aminobutyric acid (GABA) ergic input. Iontophoretically applied GABA into DRN in anaesthetised animals inhibits activity of these neurons and this effect can be reversed by application of antagonists (Gallagher and Aghajanian, 1976). Several GABAergic projections reach the DRN from widespread areas of the brain, including the ventrolateral preoptic area (VLPO), the lateral hypothalamic area, the ventral tegmental area, pontine reticular formation (PRF) (Gervasoni et al., 2000) and the DRN itself. Microdialysis perfusion of $GABA_A$ agonists (e.g. muscimol) and antagonists (e.g. bicuculline, GABAzine) into the DRN, respectively, decreased and increased 5-HT level (Tao et al., 1996; Tao and Auerbach, 2000, 2003). The $GABA_B$ agonist baclofen perfused in DRN decreased the DRN 5-HT levels, the antagonist failed to have any effect (Abellán et al., 2000; Tao et al., 1996; Tao and Auerbach, 2000). These data are consistent with the hypothesis that GABA is a source of inhibition of the DRN 5-HT cells.

Only a few studies have directly investigated the role of the GABAergic system in relation to DRN activity and sleep, and the results are not entirely consistent. Microiontophoretic application of bicuculline in DRN across the sleep–waking cycle increased the 5-HT neuron firing rate during SWS in cats, but failed to change the firing of the neurons during REM sleep and waking (Levine and Jacobs, 1992). In contrast, extracellular level of GABA were found to be significantly higher during REM sleep compared to other behavioural states (Nitz and Siegel, 1997), indicating that GABAergic cells, within or projecting to the DRN, are selectively active during REM sleep. Several workers have identified small neurons within the DRN that are more active during artificially induced REM sleep (Shiromani et al., 1995; Yamuy et al., 1995). These neurons have later been proved to be GABAergic (Torterolo et al., 2000). Microdialysis or microinjection application of GABA agonists into DRN leads to increased amounts of REM sleep, while antagonists decreased REM sleep in cats (Nitz and Siegel, 1997; Sakai and Crochet, 2001). However, in rats, bicuculline markedly increased the firing rate of DRN neurons both during waking, SWS and REM sleep (Gervasoni et al., 2000). Hence, the effect of GABA appears to be relevant throughout the sleep–wake cycle.

The aim of this study is to clarify the discrepancies related to the effect of GABA modulation on sleep and waking while simultaneously monitoring extracellular 5-HT. We hypothesise that bicuculline perfusion in DRN should increase 5-HT release in DRN and its projection sites (including REM sleep promoting areas), reduce REM sleep (McCarley et al., 1995) and possibly, affect waking and SWS. Serotonin was monitored at the site of serotonergic cells (DRN) and at a representative projection site, the frontal cortex (FC).

2. Experimental procedures

2.1. Ethical evaluation

The experiments described in this article have been approved by the local responsible laboratory animal science specialist under surveillance of the Norwegian Animal Research Authority and registered by the Authority. Norway has signed and ratified The European Convention for the protection of Vertebrate Animals used for experimental and other scientific purposes. All efforts were made to minimize the number of animal used and their suffering.

2.2. Experimental animals and surgery

Twenty-one male Sprague–Dawley (MolTac:SD) rats (Taconic M and B, Copenhagen, Denmark) weighing 200–350 g at surgery were used in these experiments. The animals were housed individually in conventional macrolone III cages. They were exposed to a 12:12 h light/dark schedule with lights on at 06:00 h. The ambient temperature averaged 22±1 °C and the relative humidity was 40–60%.

The animals had free access to food (rodent low protein diet, B&K Universal AS, Norway) and water ad libitum. To induce surgical anaesthesia animals were injected subcutaneously (s.c.) with a mixture of fentanyl, 0.05 mg/ml, fluanizone, 2.5 mg/ml, and midazolam, 1.25 mg/ml, (Hypnorm, Janssen; Dormicum, Roche). The animals received 0,05 ml/100 g of both Hypnorm and Dormicum initially, and then 0,033 ml/100 g each hour to sustain anaesthesia. The final dose ranged from 0,19 ml–0,3 ml depending on weight of the animal and length of surgery. The animals were implanted with stainless steel screw electrodes for bilateral fronto-frontal and fronto-parietal electroencephalogram (EEG) recording and silver wires in the neck muscle for electromyogram (EMG) recording (Ursin and Larsen, 1983). The frontal screw electrodes were placed