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Neuroscience Letters 172 (1994) 155–158

NEUROSCIENCE LETTERS

Blockade of thalamic $GABA_B$ receptors decreases EEG synchronization

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Received 3 December 1993; Revised version received 21 March 1994; Accepted 23 March 1994

Abstract

The γ-aminobutyric acid $(GABA)_B$ receptor antagonists 2-OH-saclofen and CGP 35348 were injected in the thalamus of freely moving cats via a microdialysis probe while recording the sleep-waking cycle. The results obtained with the two antagonists were similar: wakefulness and the total sleep time were not affected by the blockade of $GABA_B$ receptors, but deep slow wave sleep and the mean power of slow waves (<10 Hz) were decreased, while light slow wave sleep was increased. These data suggest an involvement of thalamic $GABA_B$ receptors in the regulation of EEG slow waves.

Key words: Sleep; Thalamus; $GABA_B$ receptor; Oscillation; EEG

During EEG synchronization, the firing of thalamocortical cells is characterized by low-frequency oscillations of the membrane potential often associated with high-frequency bursts of action potentials [4,17]. This pattern of activity is particularly evident in deep slow wave sleep, i.e. during delta waves, and recent investigations in vitro have demonstrated that an intrinsic oscillation with a frequency range similar to that of delta waves can be generated in thalamocortical neurones by the interplay of two voltage-dependent currents: the low-threshold Ca^{2+} current I_T, and the Na^+/K^+ inward rectifying current I_h [10,11,12,13,14].

Synaptic inputs affect the rhythmicity of this intrinsic oscillation observed in single thalamocortical neurones and of the delta waves recorded in the EEG [4]. Thus, the excitatory amino acid-mediated synaptic potentials (originating from sensory and cortical afferents), and the γ-aminobutyric acid $(GABA)_A$ receptor mediated synaptic potentials (originating from cells of the nucleus reticularis thalami and from thalamic interneurones) advance or delay the phase of the oscillation and can even abolish it [16]. The $GABA_B$ receptor-mediated IPSPs might also contribute to the generation of low-frequency oscillations and associated burst firing of thalamocortical cells observed in vivo and in vitro, since the properties of these potentials (e.g. non-linear relationship with membrane potential, duration, rate of decay, etc.) are highly suited for the activation of I_T [5].

In order to investigate directly the role of thalamic $GABA_B$ receptors in sleep and EEG synchronization, 2-hydroxy-saclofen (2-OH-saclofen) and CGP 35348, two selective antagonists of $GABA_B$ receptors [1,3,15], were injected via a microdialysis probe into the ventro-posterolateral nuclei of the thalamus of freely moving cats. This technique allows the continuous and localized application of competitive receptor antagonists that is necessary to induce behaviouraly detectable effects on a long-lasting event such as sleep [7,8]. We report that blockade of thalamic $GABA_B$ receptors decreases deep slow wave sleep and the mean power of low-frequency (< 10 Hz) EEG waves, and increases light slow wave sleep.

The experiments were performed on 14 freely-moving, adult cats (3–4 kg) following the procedures described in detail in previous studies [7,8,9]. Briefly, under Nembutal anaesthesia, dental screw electrodes were implanted into the skull above the occipital and frontal cortex to record the EEG. Two electrodes were implanted into the orbital cavity for recording the electrooculogram (EOG), and stainless steel electrodes were placed into the neck muscles to record the electromyogram (EMG). Microdialysis

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SSDI 0304-3940(94)00245-6

probes, made of hollow fibers (Travenol) of 5000 Da cutoff point and with an active surface of 3 mm and an outside diameter of 0.2 mm were implanted into the ventroposterolateral thalamic nuclei [8]. Antiseptic and local anaesthetic treatment were used during these procedures [8].

The animals were housed into a sound proof and electrically shielded recording cage to which they had been habituated for a week before implantation. They were supplied with food 30 min before starting the recording session. During the recording session (4 h, from 9:00 am to 1:00 pm) the EEG, EOG and EMG were recorded with an 8 channel recorder (EEG 8S, Medicor), and 4 fronto-occipital leads were also connected to a computer (via a 1401 interface, CED, Cambridge, UK). Each data block (10 s duration) was digitized at 100 kHz using an EEG analysis software (CED), and the power spectra of the EEG was calculated on-line and displayed in waterfall mode. The selected frequency bands were 0–5 Hz, 5–10 Hz, 10–20 Hz. The stages of vigilance were differentiated into four stages by analysis of the EEG, EOG and EMG recordings according to standard polygraphic criteria: wakefulness, when the muscular tone was high and the EEG was permanently desynchronized; light slow wave sleep, when sleep spindles were present in the EEG and the power of delta waves was not stable; deep slow wave sleep, when the power of the delta wave frequency range (< 5 Hz) in 10 consecutive power spectra (100 s total time) was stable (< 10% variation); and paradoxical sleep, in which the muscular tone dropped to zero, the EEG was desynchronized and rapid eye movements were observed in the EOG.

Because the formation of glyosis around the microdialysis probes (even in the presence of cytosine arabinose, see below) [7,8] limits their optimal performance to a few days after implantation, sleep recording was initiated on the third day following implantation. In order to decrease the variability caused by spontaneous changes in sleep stages following the short recovery period, two control recording sessions were carried out, one the day before and the other the day after the drug treatment [8]. The results obtained in these two control days were then averaged and used as the control value, though in each animal no significant difference was observed in the mean power of the EEG frequency bands and in the number and duration of each stage of the sleep–waking cycle between these two control days [7,8,9]. Artificial cerebrospinal fluid (containing 147 mM NaCl, 3.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.01 mM cytosine arabinose, pH 7.3) was applied at a rate of 2 μl/min through the microdialysis probes in the control days. The $GABA_B$ receptor antagonist 2-OH-saclofen (Tocris Neuramin, UK) and CGP 35348 (kindly supplied by Ciba-Geigy, Basel) were added to this perfusion solution at a concentration of 3 mM (10 animals) and 1 mM (4 animals), respectively. Since the concentration in the proximity of the dialysis probe is about 10% of that inside the probe [8], the effective concentration of the two antagonists was 0.3 mM and 0.1 mM, respectively. The animals were only perfused during the recording session. At the end of the experiments (generally, 6–9 days after implantation) the animals were deeply anaesthetised with a high dose of Nembutal and transcardialy perfused with saline. The position of the dialysis probe was then verified on Nissl-stained sections. Results are expressed in the text and figure as mean ± S.E.M., and statistical analysis was performed by Student's *t*-test.

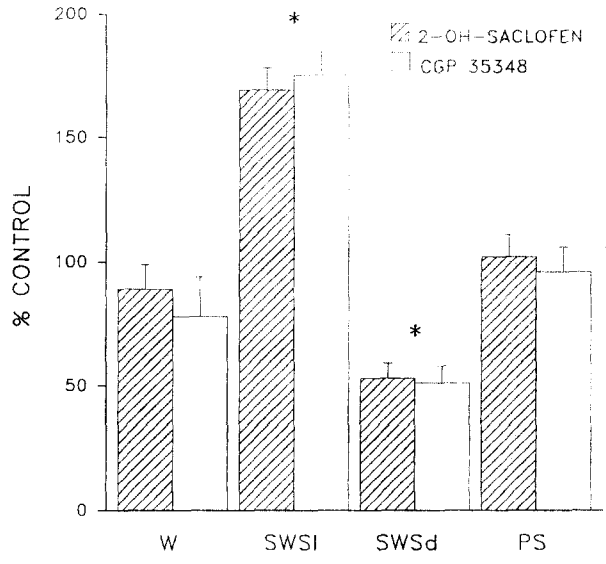

Fig. 1. The effect of 2-OH-saclofen (0.3 mM) and CGP 35348 (0.1 mM) on the sleep–waking cycle. The histogram show the duration of each stage of the sleep–waking cycle expressed as percentage of that in control days. 2-OH-saclofen and CGP 35348 increased light slow wave sleep (SWSI) and decreased deep slow wave sleep (SWSd), but had no effect on wakefulness (W) and paradoxical sleep (PS). See text for significance values.

The continuous application of 2-OH-saclofen (0.3 mM) or CGP 35348 (0.1 mM) for 4 h did not produce any pathological change in the EEG nor any modification in the behavioural responsiveness of the animals, indicating the absence of a myorelaxant effect and of any major non-specific actions due to accumulation of the two drugs. The amount of wakefulness and the total sleep time were unaffected by the $GABA_B$ antagonists (Fig. 1), so that the sleep/wakefulness ratio (2-OH-saclofen: 4.7 ± 1.6; CGP 35348: 4.3 ± 0.9) was identical to that in control days (4.3 ± 0.6). However, the amount of light slow wave sleep increased by 69 ± 9% ($P < 0.05$) and 75 ± 11% ($P < 0.05$), while that of deep slow wave sleep decreased by 47 ± 6% ($P < 0.05$) and 49 ± 7% ($P < 0.001$), respectively (Fig. 1). Thus, the ratio light/deep slow wave sleep was enhanced from 0.8 ± 0.1 in control to 3.2 ± 0.1 ($P < 0.01$) during 2-OH-saclofen, and to 3.4 ± 0.2 ($P < 0.05$) during CGP 35348. No

change occurred in the amount of paradoxical sleep, though it was observed that episodes of paradoxical sleep often started after shorter periods of deep slow wave sleep than in control days.

To investigate in more detail the effect of blockade of $GABA_B$ receptors, the EEG power spectra density was analyzed using a total of 1500 s of EEG recording for each stage of the sleep-waking cycle in the animals that received 2-OH-saclofen. In wakefulness and paradoxical sleep no change was observed in the three frequency bands (0–5, 5–10 and 10–20 Hz) during application of this $GABA_B$ antagonist. In light slow wave sleep, however, a statistically significant decrease was found in the mean power of the 0–5 Hz frequency band ($19.7 \pm 4.9\%$, $P < 0.005$) and of the 5–10 Hz frequency band ($23.7 \pm 5.8\%$, $P < 0.01$) in the presence of 2-OH-saclofen. No change in the EEG spectra above 10 Hz was observed both in light and deep slow wave sleep.

2-OH-saclofen and CGP 35348 have been shown to block thalamic $GABA_B$ receptors reversibly [6,15]. Application of these antagonists into the ventroposterolateral nuclei of the thalamus did not change the sleep/wakefulness ratio, but decreased the amount of deep slow wave sleep and the power of slow waves (< 10 Hz), and increased light slow wave sleep. The lack of any major behavioural change during blockade of thalamic $GABA_B$ receptors and the similarity in the effects obtained with the two $GABA_B$ antagonists indicate that their action on the sleep-waking cycle was not due to nonspecific actions. Thus, the present data support an involvement of thalamic $GABA_B$ receptors in synchronized EEG waves in the intact animal.

When changes in the duration of more than one sleep stage occur following pharmacological treatment, it is often difficult to differentiate between the primary and the secondary effect [8], i.e. between light and deep slow wave sleep in the case of the present results. Some indication, however, can be obtained by considering the changes in the duration of the different sleep stages together with the changes in the EEG power spectra. In this way, the most parsimonious explanation of our data is that a decrease in the power spectra of the 0–5 Hz frequency bands represents the primary effect produced by $GABA_B$ receptor blockade. This reduction, in fact, would result in a decreased duration of deep slow wave sleep, and thus the increase in light slow wave sleep might be considered as a consequence of a decreased deep slow wave sleep. The alternative possibility (i.e. that the primary effect of $GABA_B$ receptor blockade is the reduction in the power spectra of the 5–10 Hz frequency band) would support a decrease of light slow wave sleep, and not an increase as it was observed in this study.

The inability of 2-OH-saclofen and CGP 35348 injected into the thalamus to abolish EEG synchronization indicates either a limited spread of the antagonists from the injection site (but see ref. 8 for the effect produced by thalamic application of excitatory amino acid antagonists), and/or that thalamic $GABA_B$ receptors do not play an exclusive role in the generation of low-frequency EEG waves. Indeed, as explained in the introduction, thalamocortical cells have the ability of generate intrinsic oscillations in the frequency range of delta waves via voltage-activated membrane currents [10,11,12,13,14].

While pre- and post-synaptic $GABA_B$ receptors have been characterized in other CNS regions [1,2], only the post-synaptic receptors have so far been extensively investigated in the thalamus [5,15]. Preliminary electrophysiological results in vitro, however, indicate that presynaptic $GABA_B$ receptors are also present in dorsal lateral geniculate nucleus both on excitatory and inhibitory terminals, where they appear to exert a negative control over glutamate and GABA release, respectively [6,15]. Although the weak potency of low concentrations of the antagonists on pre-synaptic $GABA_B$ receptors [1,2] indicates that the effects reported in the present study might involve post-synaptic $GABA_B$ receptors, any firm conclusion as to whether the effects of 2-OH-saclofen and CGP 35348 described in this study are mediated by pre- and/or post-synaptic $GABA_B$ receptors cannot be drawn at present.

This work was supported by Grant OTKA/628 (from the Hungarian Academy of Sciences), Grants 32491/1.15 and 19135/1.15 (from the Wellcome Trust), and by an Hungarian Science Aid grant (from the Hungarian Credit Bank to A.K. Kekesi).

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