European Journal of Pharmacology, 209 (1991) 131–133

EJP 20976

Short communication

Sleep promoting effect of a putative glial γ-aminobutyric acid uptake blocker applied in the thalamus of cats

G. Juhász [1], K.A. Kékesi [1], Zs. Emri [1], J. Ujszászi [2], P. Krogsgaard–Larsen [3] and A. Schousboe [4]

[1] *Department of Comparative Physiology, Eötvös Loránd University, Budapest, Hungary,* [2] *National Institute of Mental Health, Budapest, Hungary,* [3] *PharmaBiotec Research Center, Department of Organic Chemistry, Royal Danish School of Pharmacy and* [4] *PharmaBiotec Research Center, Department of Biochemistry A, Panum Institute, University of Copenhagen, Copenhagen, Denmark*

Received 9 October 1991, accepted 29 October 1991

The uptake of γ-aminobutyric acid (GABA) by glial cells was decreased when 4,5,6,7,-tetrahydroisoxazolo-(4,5-C)-pyridin-3-ol (THPO) was applied in the thalamus of freely moving cats by in vivo microdialysis. A marked reduction in duration of wakefulness and in number of awakenings was obtained during THPO treatment. THPO did not change the ratio of slow-wave-sleep and paradoxical sleep but only increased the total sleep time. The present data suggest a possible regulatory role of the glial–neuronal interaction in the modification of the sleep-waking cycle.

GABA (γ-aminobutyric acid); Glial uptake; Thalamus; Sleep; (Cat)

1. Introduction

The increase in efficacy of GABA (γ-aminobutyric acid)ergic transmission by benzodiazepines (Scherschlicht and Pieri, 1988) or by sustained application of GABA into the extracellular fluid (Juhász et al., 1989a) promotes sleep. Because inhibition of the sensory input is a prerequisite of sleep (Steriade and Llinas, 1988) and most of the sensory interneurons are GABAergic (Jones, 1985), the sleep-promoting effect of the GABAergic neurons could be due at least in part to increased inhibition of the excitatory amino acidergic neurons in the thalamus. The first stage of the sensory pathway where information transmission is reduced preceding sleep onset is located in the thalamus (Steriade and Llinas, 1988). The transmitter of the thalamic relay cells is likely to be glutamate (Jones, 1985), and the interneurons are GABAergic (Jones, 1985). A number of GABAergic terminals are clustered in the thalamic synaptic glomeruli which are covered by a glial cell sheet (Jones, 1985). The sensory input to the thalamic relay cells also arrives mainly through synapses of the synaptic glomeruli, suggesting that the sleep-related decrease in thalamic information transmission involves glomerular mechanisms to some extent. In the thalamus, the amino acidergic cells themselves might be able to promote synchronization and sleep without involvement of other transmitter-specific systems (Juhász et al., 1990).

Evidence supports the regulation of amino acid transmitter pools in the neurons by the glial–neuronal interaction (Gonsalves et al., 1989). It is assumed that one of the major roles of the glial cells is to remove the excess of transmitters from the extracellular space following synaptic activity. Hence, inhibition of glial GABA uptake could lead to an increase in the extracellular GABA concentration. In line with this, it has been shown that the glial GABA uptake inhibitor (Schousboe et al., 1981), 4,5,6,7,-tetrahydroisoxazolo-(4,5-C)-pyridin-3-ol (THPO) protects against seizure activity generated by a deficit in GABAergic transmission (Gonsalves et al., 1989a). This suggests that the glial GABA uptake system possibly makes a major contribution to the modulation of GABAergic transmission. Further, it seems plausible that the decrease in sensory information transmission in the thalamus, which is a prerequisite of sleep (Steriade and Llinas, 1988), is mainly due to the enhanced GABAergic influences. The question then arises as to whether a decrease in the GABA uptake in the glial cells promotes sleep.

A specific and local decrease in the glial GABA uptake may be achieved by sustained microdialysis application of THPO into the extracellular space of the thalamus. This technique allows chronic perfusion of

Correspondence to: G. Juhász, Department of Comparative Physiology, Eötvös Loránd University, Múzeum krt. 4/a, Budapest 1088, Hungary. Fax 36.1.1181 154.

particular compartments of the brain without the injection of solvents directly into brain tissue (Marsden, 1984). In the present study, THPO was perfused for 4 h in freely moving cats and the effect of this perfusion on the sleep-waking cycle was recorded.

2. Materials and methods

The measurements were carried out on four adult cats. They were anesthetized with Nembutal (40 mg/kg i.p.) and electrodes were implanted into the skull for polygraphic recording of sleep stages. Dialysis probes, made of Travenol dialysis fibers (200 μm outer diameter) in which two glass capillaries were inserted, were implanted into the ventro-posterolateral thalamic nuclei (VPL, A: 9, L: 7, V: −1). The distance between the tips of the capillaries was 3 mm. The inlet and outlet tubes of the dialysis probes were connected to a standard chromatographic tube connector cemented to the skull. The transport properties and the dead volume of the samplers were measured in vitro by an electrochemical microcell system as described previously (Juhász et al., 1989). The in vitro outflow of 0.005 M/ml THPO from the microdialysis probes was 10%. Consequently the THPO transported into the extracellular space of the VPL was 0.000062 M/mm^2 × min at a flow rate of 2 μl/min, which was found to be a sufficient dosage of THPO for initiation of changes in the sleep of the cats.

Following implantation, the dialysis probes were perfused with artificial cerebrospinal fluid (ACSF: pH 7.3, Na^+ 140 mEq, K^+ 3 mEq, Mg^{2+} 1 mEq, Ca^{2+} 1 mEq) for 4 h each day. The perfusion solution contained 10–6 M cytosine arabinoside to reduce the development of a glial barrier around the sampler (Juhász et al., 1989b). The sleep stages were differentiated by the standard polygraphic criteria (Moruzzi, 1972) and recording was performed for 4 h each day from 09:00 to 13:00 h. The stages of vigilance were differentiated into four phases by visual analysis of polygraph recordings: wakefulness (W), slow-wave-sleep light phase (SWSl), slow-wave-sleep deep phase (SWSd) and paradoxical sleep (PS). SWSl and SWSd were differentiated on the basis of the continuous appearance of delta waves in the electroencephalogram (EEG), measured with EEG analysis software (Cambridge Electronic Devices). The number of miniature awakenings (shorter than 0.3 s) (Naitok et al., 1982) was also tested. The application of THPO in the perfusion solution was preceded and followed by control sessions when ACSF was injected. The relative duration of various stages of the sleep-waking cycle recorded during these two control days was averaged and compared (Student's t-test) with the results obtained during the application of THPO. The results were expressed as percentages of the control values, thus large relative decreases in W caused only slight relative increases in other stages. The arrangement of control and THPO-treated sessions was intended to eliminate the spontaneous shifts in the sleep-waking cycle following implantation. The position of the sampler was checked by histological evaluation of Nissl-stained sections.

3. Results

Intrathalamic application of THPO produced a highly significant reduction in W (fig. 1). A more detailed analysis of the sleep profile showed that the reduction in W during application of THPO was due mainly to a decrease in the frequency of W episodes (fig. 2A) while their duration was unchanged. The application of THPO did not significantly change either SWSl, SWSd or PS due to the relatively small amount of W compared to sleep. THPO treatment did not change the number of miniature awakenings shorter than 0.3 min (fig. 2B). There were no changes in EEG power and spectral distribution of EEG waves during THPO application.

4. Discussion

The application of THPO by microdialysis allowed the localized and sustained perfusion of THPO into a thalamic relay nucleus. However, it should be noted that the exact concentration of THPO around the dialysis probe was not known. The relative outflow can be estimated on the basis of an in vitro calibration (Juhász et al., 1989b) but this estimation is not necessarily valid in the brain.

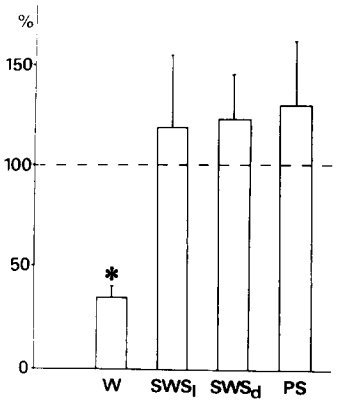

Fig. 1. Changes in wakefulness (W), slow-wave-sleep light phase (SWSl), slow-wave-sleep deep phase (SWSd) and paradoxical sleep (PS) during application of the selective glial GABA uptake blocker, THPO, relative to the control values taken as 100%. The asterisk indicates a statistically significant change at $P < 0.001$ level ($N = 4$).

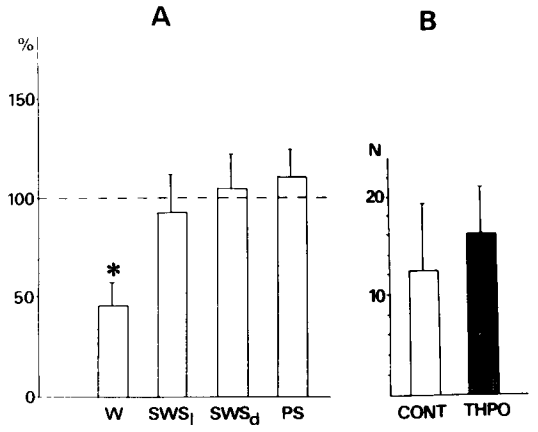

Fig. 2. (A) Changes in the average frequency of sleep episodes during the 4-h recording session. Numbers of sleep stages in the control sessions, 100%. (B) Effect of THPO on the number of miniature awakenings. The asterisk indicates a statistically significant change at the $P < 0.001$ level.

THPO reduced the number of W episodes but did not influence the number of miniature awakenings. The decrease in the incidence of sustained spontaneous waking episodes suggests that THPO promotes the generation of synchronous EEG activity in the thalamus by increasing the GABA 'signals'. However, the absence of effects on miniature awakenings indicates that THPO might have little or no effect on the signals coming into the thalamus from the brainstem or from the sensory pathways (Naitok et al., 1982). Further investigations of THPO-induced changes in sensory information transmission through the thalamic relay cells therefore seem necessary.

Similar to the effects of benzodiazepines and GABA, local application of THPO into the VPL caused a reduction in W. The inhibition of GABA uptake into glial cells of the thalamus might result in an increase in the extracellular concentration of GABA under the astrocyte cover of the thalamic synaptic buttons, leading to a prolonged GABA 'signal'. On the basis of the present study, it appears that glial uptake of GABA probably plays a role in the modulation of thalamic neurons controlling the sleep-waking cycle.

References

Gonsalves, S.F., B. Twitcell, R.E. Harbaugh, P. Krogsgaard-Larsen and A. Schousboe, 1989, Anticonvulsant activity of intracerebroventricularly administered glial GABA uptake inhibitors and other GABA mimetics in chemical seizure models, Epilepsy Res. 4, 34.

Jones, E.G., 1985, The Thalamus (Plenum, New York).

Juhász, G., Zs. Emri, K.A. Kékesi and K. Pungor, 1989a, Local perfusion of the thalamus with GABA increases sleep and induces long-lasting inhibition of somatosensory event-related potentials in cats, Neurosci. Lett. 103, 229.

Juhász, G., J. Tarcali, K. Pungor and E. Pungor, 1989b, Electrochemical calibration of in vitro brain dialysis samplers, J. Neurosci. Meth. 103, 131.

Juhász, G., K.A. Kékesi, Zs. Emri, I. Soltész and V. Crunelli, 1990, Sleep-promoting action of excitatory amino acid antagonists: a different role for thalamic NMDA and nonNMDA receptors, Neurosci. Lett. 114, 333.

Marsden, C.A., 1984, Measurement of Transmitter Release (Wiley, London).

Moruzzi, G., 1972, The sleep-waking cycle, Ergebn. Physiol. 64, 1.

Naitok, P., V. Antony-Baas, A. Muzet and J. Ehrhart, 1982, Dynamic relation of sleep spindles and K-complexes to spontaneous phasic arousal in sleeping human subjects, Sleep 5, 58.

Scherschlicht, A. and L. Pieri, 1988, Pharmacology of midazolam and other short acting benzodiazepines, in: Sleep 86, eds. W.R. Koella, F. Obal, H. Schulz and P. Visser (Fischer, Stuttgart) p. 101.

Schousboe, A., O.M. Larsson, L. Hertz and P. Krogsgaard-Larsen, 1981, Heterocyclic GABA analogues as new selective inhibitors of astroglial GABA transport, Drug Dev. Res. 1, 115.

Steriade, M. and R.R. Llinas, 1988, The functional states of the thalamus and the associated neuronal interplay, Physiol. Rev. 68, 649.