Neuroscience Letters, 114 (1990) 333–338
Elsevier Scientific Publishers Ireland Ltd.

NSL 06966

Sleep-promoting action of excitatory amino acid antagonists: a different role for thalamic NMDA and non-NMDA receptors

Gábor Juhász[1], Katalin Kékesi[1], Zsuzsa Emri[1], Ivan Soltesz[2] and Vincenzo Crunelli[2]

[1]*Department of Comparative Physiology, Eötvös Loránd University, Budapest (Hungary) and* [2]*Department of Pharmacology, St. George's Hospital Medical School, London (U.K.)*

(Received 5 January 1990; Revised version received 3 March 1990; Accepted 8 March 1990)

Key words: Sleep; Thalamus; Glutamate; *N*-Methyl-D-aspartate receptor; Brain dialysis

Changes in the sleep–waking cycle of freely moving cats were studied during application of excitatory amino acid antagonists in the ventro-posterolateral thalamic nuclei by microdialysis. DL-2-Amino-5-phosphono-pentanoic acid (APV), a selective *N*-methyl-D-aspartate (NMDA) receptor antagonist, produced an increase in the deep stages of slow wave sleep and in paradoxical sleep and a decrease in the light stages of slow wave sleep (SWS_1), while 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), at a concentration selective for the non-NMDA receptors, produced a marked increase in SWS_1. These results indicate a strong sleep-promoting action of excitatory amino acid antagonists and suggest that thalamic NMDA and non-NMDA receptors may play different roles in sleep regulation. Thus, changes in the sleep–waking cycle should be carefully evaluated when assessing the potential clinical use of excitatory amino acid antagonists.

A decrease in the sensory input to the brain can induce sleep [1, 15, 20] and the thalamus is the first brain area along the sensory pathways where the transmission of information is reduced at the sleep onset [20]. Numerous evidence has indicated that the sensory (as well as the cortical) input to the majority of the thalamic nuclei is mediated by excitatory amino acid(s) via activation of both *N*-methyl-D-aspartate (NMDA) and non-NMDA (i.e. quisqualate and kainate) receptors [6, 7, 12, 16, 17]. We now report that both DL-2-amino-5-phosphono-pentanoic acid (APV), a selective NMDA receptor antagonist [23], and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) [9], used at a concentration selective for the non-NMDA receptors [3, 18], applied into the ventro-posterolateral thalamic nuclei (VPL) of freely moving cats by microdialysis [10, 11, 14], increase sleep. APV produces an increase in the deep stages of slow wave sleep (SWS_d) and in paradoxical sleep (PS) and a decrease in the light

Correspondence: V. Crunelli. Present address: Department of Visual Science, Institute of Ophthalmology, Judd Street, London WC1H 9QS, U.K.

stages of slow wave sleep (SWS_I), while the major effect of CNQX is an increase in SWS_I.

Experiments were performed on 8 adult cats (2.3–3.4 kg) as described previously [10, 11]. Under Nembutal anaesthesia (50 mg/kg i.p.), gold-plated screw electrodes were implanted in the frontal and occipital cortex for electroencephalographic (EEG) recordings and in the orbital cavity for electrooculographic (EOG) recordings, while stainless-steel electrodes were inserted into the neck muscles to record the electromyogram (EMG). Two microdialysis probes [10, 11] were implanted bilaterally into the VPL [A: 9, L: 7, V: 0–3) [2]. After implantation, the animals were allowed to recover in their moderately light home-cage which was placed in a sound- proof and electrically shielded room. During this period they were habituated to the recording cables and the connecting tubes which were perfused with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 142, $MgCl_2$ 2.2, $CaCl_2$ 2.3, KCl 2.8 (pH 7.3) and cytosine arabinoside (0.01 μM) to reduce formation of gliosis around the dialysis probe [10, 11].

Sleep recording was initiated on, or after, the third day following implantation and consisted of a 4 h daily session, from 09.00 to 13.00 h, 1 h after feeding. The stages of vigilance were differentiated into 4 phases by visual analysis of the EEG, EMG and EOG recordings and according to the standard polygraphic criteria [15]: wakefulness (W), SWS_I, SWS_d and PS. The injection of each excitatory amino acid antagonist was preceeded and followed by a day in which ACSF was injected. The various stages of the sleep–waking cycle recorded during these two 'control' days were averaged and compared with the results obtained during the injection of the excitatory amino acid antagonist. At the end of the experiments (generally 10–12 days after implantation) the animals were injected with a high dose of Nembutal and transcardially perfused with saline. The positions of the dialysis probes were then verified on Nissl-stained sections.

The preparation and use of the microdialysis probes have been described in detail elsewhere [11]. Briefly, they were made by inserting two glass capillaries into a Travenol hollow fibre (outside diameter: 0.2 mm) with a 3 mm distance between their tips, thus providing a 3 mm long active surface. Thus, according to the size of the dialysis probe, the injection of APV and CNQX was performed around a cylinder shaped area, 3 mm long and 0.2 mm wide. The amount of excitatory amino acid antagonist pumped out of the dialysis probe was calculated by comparison with dopamine [11]. In fact, since at a certain concentration gradient the transport of molecules through the dialysis membrane depends only on their molecular weight, compounds with similar molecular weights have similar outflows from the probe (molecular weights: dopamine 190, APV 197, CNQX: 249). We estimated an outflow of 9 pg/mm^2/min for APV and CNQX when present in μM concentration in the perfusion solution. Consequently, 500 μM APV or 100 μM CNQX was added to the ACSF providing a concentration of 50 μM APV and 10 μM CNQX near the dialysis probe. Since the active surface of the dialysis probe is about 0.1 mm^2, the estimated total flux of APV and CNQX was 0.9 pg/min. Numerical data are reported in the text as mean $\pm$ S.E.M. and Student's t-test for paired values was used for statistical analysis.

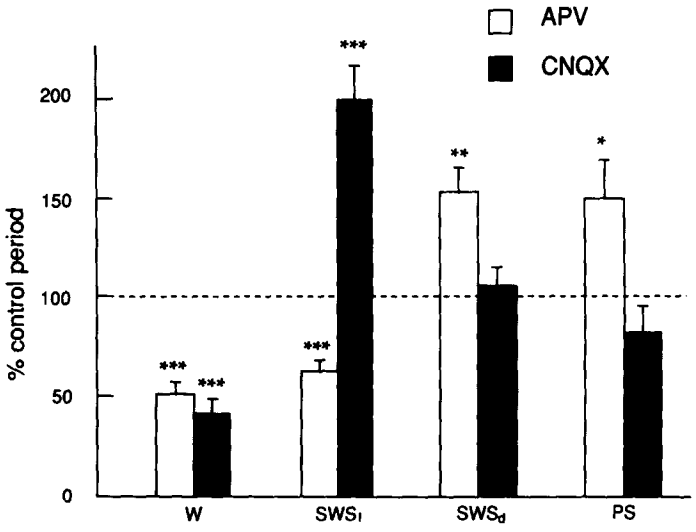

Fig. 1. Effect of 2-amino-5-phosphono-pentanoic acid (APV, □) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, ■) on different stages of the sleep–waking cycle. Results are expressed as percentage of the control days (see text) when ACSF was injected ($n=8$, $*P<0.05$; $**P<0.01$, $***P<0.001$, in respect to the corresponding stage during the control days, Student's t-test). (W, wakefulness; SWS_I, light phase of slow wave sleep; SWS_d, deep phase of slow wave sleep, PS, paradoxical sleep).

Intrathalamic application of APV produced a statistically significant reduction in W and SWS_I and an increase in SWS_d and in PS (Fig. 1). Thus, the sleep/W ratio increased (control: 4.7 ± 1.2, APV: 10.8 ± 1.9, $n=8$, $P<0.05$) and the SWS_I/SWS_d ratio decreased (control: 0.7 ± 0.1, APV: 0.3 ± 0.1, $n=8$, $P<0.005$). APV also decreased the latency of the first PS episode (control: 54 ± 10 min, APV: 26 ± 9 min, $n=8$, $P<0.06$). In two animals, a 50% reduction in the concentration of APV produced similar, though smaller, changes in the various stages of the sleep–waking cycle. A more detailed analysis of the sleep profile showed that the reduction in W during application of APV was due mainly to a decrease in the number of W episodes while their duration was unchanged (Table I) On the contrary, the increase in SWS_d was mainly due to an increase in the duration (but not the number) of the individual SWS_d episodes (Table I). Probably because of small changes in both their number and duration, the individual episodes of SWS_I and PS did not show any statistical significance.

The intrathalamic application of CNQX also decreased W (Fig. 1). The effect of CNQX on the sleep pattern, however, was different from the effect of APV. CNQX markedly enhanced SWS_I but had no effect on SWS_d, PS (Fig. 1) or the latency of the first PS episode. Thus, the SWS_I/SWS_d ratio almost doubled from 0.7 ± 0.1 in the control period to 1.3 ± 0.2 during CNQX application ($n=8$, $P<0.01$). This effect of CNQX was due to a decrease in the number of W episodes (but not their duration) and an increase in the duration (but not the number) of the individual SWS_I episodes (Table I). Note that no change in the behaviour and body temperature of the animals was observed during or following the application of either antagonist which had no

TABLE I

EFFECT OF 2-AMINO-5-PHOSPHONO-PENTANOIC ACID (APV) AND 6-CYANO-7-NITRO-QUINOXALINE-2,3-DIONE (CNQX) ON THE NUMBER AND DURATION (min) OF INDIVIDUAL EPISODES OF THE DIFFERENT SLEEP STAGES

Values represent mean ± S.E.M. $n = 8$. W, wakefulness; SWS_l, light phase of slow wave sleep; SWS_d, deep phase of slow wave sleep; PS, paradoxical sleep.

	Control	APV	CNQX
W			
Number	11.1 ± 0.8	8.1 ± 0.8*	5.8 ± 0.8***
Duration	6.3 ± 1.1	3.6 ± 0.8	5.5 ± 1.3
SWS_l			
Number	11.3 ± 0.9	10.1 ± 1.1	11.9 ± 1.0
Duration	5.4 ± 0.4	4.4 ± 0.7	8.7 ± 1.2**
SWS_d			
Number	12.1 ± 0.8	14.3 ± 1.9	10.4 ± 0.8
Duration	8.1 ± 0.5	11.6 ± 1.7*	9.6 ± 1.1
PS			
Number	5.5 ± 0.5	8.5 ± 1.0	5.4 ± 0.7
Duration	6.6 ± 0.6	6.1 ± 0.7	6.8 ± 1.1

*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$.

myorelaxant activity. In addition, although the exact concentration and diffusion of the two antagonists around the probe are not known, the different effects of APV and CNQX on the sleep–waking cycle, the dose-dependent action of APV and the lack of any consistent behavioural modification suggest the absence of major non-specific actions due to accumulation of the antagonists.

Other drugs, structurally different from the antagonists tested in this study but all known to affect the sleep–waking cycle in experimental animal and in man, have been recently shown to interfere with excitatory amino acid mediated transmission. Thus, ketamine, a dissociative anaesthetic, has been shown to inhibit excitatory postsynaptic potentials (EPSPs) and NMDA mediated responses in the cortex [22] and thalamus [5, 18] in vitro, while dextromethorphan, an antitussive drug of common clinical use that has recently been shown to block NMDA receptors in vitro [4, 24], is known to induce drowsiness in man [8]. Furthermore, oral administration of Riluzole has been reported to promote sleep in rats [21], though the precise mechanism by which this substance affects excitatory amino acid mediated synapses is still controversial. The present results, however, represent the first direct evidence linking the two different classes of excitatory amino acid receptors mediating sensory-thalamic and cortico-thalamic inputs to the sleep–waking cycle.

The different effects of APV and CNQX on the sleep–waking spectra suggest a different role for thalamic NMDA and non-NMDA receptors in the sleep–waking cycle. However, although the relative contribution of the NMDA and non-NMDA

component to sensory and cortical EPSPs in the thalamus appear to be different [6, 7, 12, 16, 17], it is rather difficult, at present, to assign a precise role/function in the sleep–waking cycle to each of the two types of excitatory amino acid receptors. A likely explanation for the increase in SWS_l by CNQX might be that the reduction by this antagonist of the sensory (and cortical) EPSPs in thalamocortical cells will result in a decreased input to the cells of the nucleus reticularis thalami [20], thus allowing this latter group of cells to express their rhythmic burst behaviour [19, 20] which has been suggested to be responsible for spindle activity [19, 20].

The interpretation of APV action is complicated by the fact that this antagonist affected both SWS_l, SWS_d and PS. The increase in PS, for instance, might suggest that thalamic NMDA receptors are directly involved in the desynchronized activity of the EEG characteristic of PS. However, it is more likely that the increase in PS following blockade of NMDA receptors might simply be the consequence of the increased SWS_d since the latter is known to be a prerequisite of PS [15, 20]. As far as SWS_l and SWS_d are concerned, it is possible that NMDA receptors are directly involved in the regulation of both, or only in one of them, with the other sleep stage changing as an indirect effect. Recent experiments in vivo and in vitro tend to favour the latter possibility, i.e. that a reduced NMDA receptor stimulation is the main contributing factor involved in the deepening of SWS and generation of δ waves in the EEG, while the decrease in SWS_l simply represents a consequence of this direct action. In fact, thalamocortical cells in vitro are capable of a slow (2–4 Hz), spontaneous burst firing which is independent of γ-aminobutyric acid (GABA), acetylcholine, noradrenaline and non-NMDA receptor activation [5, 13]. The block of NMDA receptors changes this pattern of activity into a highly rhythmic, 'pacemaker-like' firing with a concomitant decrease in frequency from 2 to 4 Hz to values similar to those characteristic of the deepest stages of SWS (0.5–2.5 Hz) [13]. Interestingly, a similar, slow (2 Hz), highly rhythmic, burst firing has also been recorded in vivo in thalamocortical cells separated from the sensory, cortical and reticularis thalami inputs [19, 20]. Thus, the increase in SWS_d by APV observed in this study might be explained as if the selective blockade of thalamic NMDA receptors, by releasing thalamocortical cells from the NMDA component of sensory and cortical EPSPs, is allowing these cells to express their intrinsic pattern of spontaneous activity, i.e. that observed during the deepest stage of SWS in vivo or the 0.5–2 Hz 'pacemaker-like' firing in vitro.

In conclusion, intrathalamic application of APV or CNQX, two potent and selective excitatory amino acid antagonists, reduced W and promoted sleep. This finding strongly suggests that both NMDA and non-NMDA receptor mediated inputs to the thalamus play a major and different role in sleep regulation. Furthermore, whatever the precise role of NMDA and non-NMDA receptors in sleep regulation, the present results warrant that changes in the sleep–waking cycle should be carefully evaluated when assessing the potential clinical use of excitatory amino acid antagonists.

We wish to thank Dr. N. Leresche and Mr. S. Lightowler for their helpful suggestions and critical comments. The work was supported by the Hungarian Academy of Sciences (Grants OKKFT3630313 and OTKA380013) and by the Wellcome Trust (Grants 17953/1.5 and 19135/1.5). I.S. was funded by the Herbert von Karajan Neu-

roscience Research Trust and acknowledges support from the Neurobiology Research Laboratory of the Hungarian Academy of Sciences.

1 Andersen, P. and Andersson, S.A., Physiological Basis of the Alpha Rhythm, Appleton-Century-Crofts, New York, 1968.
2 Bermann, A.L., The Brainstem of the Cat, University of Winsconsin Press, Madison, 1968.
3 Blake, J.F., Brown, M.W. and Collingridge, G.L., CNQX blocks acidic amino acid induced depolarizations and synaptic components mediated by non-NMDA receptors in rat hippocampal slices, Neurosci. Lett., 89 (1988) 182–186.
4 Church, J., Lodge, D. and Berry, S.C., Differential effects of dextrorphan and levorphanol on the excitation of rat spinal neurons by amino acids, Eur. J. Pharmacol., 111 (1985) 185–190.
5 Crunelli, V., Haby, M., Jassik-Gerschenfeld, D., Leresche, N. and Soltesz, I., NMDA receptors and rhythmic membrane potential oscillations in rat and cat thalamic projection cells in vitro, J. Physiol. (Lond.), 406 (1988) 12P.
6 Crunelli, V., Kelly, J.S., Leresche, N. and Pirchio, M., On the excitatory postsynaptic potential evoked by stimulation of the optic tract in the lateral geniculate nucleus, J. Physiol. (Lond.), 384 (1987) 603–618.
7 Deschenes, M. and Hu, B., Electrophysiology and pharmacology of the corticothalamic response, Soc. Neurosci. Abstr., 15 (1989) 374.11.
8 Goodman-Gilman, A., Goodman, L.S. and Gilman, A., The Pharmacological Basis of Therapeutics, Macmillan, London, 1987.
9 Honore, T., Davies, S.N., Drejer, J., Fletcher, E.J., Jacobsen, P., Lodge, D. and Neilsen, P.E., Quinoxalinediones: potent competitive non-NMDA receptor antagonists, Science, 241 (1988) 701–703.
10 Juhasz, G., Emri, Z., Kekesi, K. and Pungor, K., Local perfusion of the thalamus with GABA increases sleep and induces long-lasting inhibition of somatosensory event-related potentials in cats, Neurosci. Lett., 103 (1989) 229–233.
11 Juhasz, G., Tarcali, J., Pungor, K. and Pungor, E., Electrochemical calibration of in vivo brain dialysis samplers, J. Neurosci. Methods, 29 (1989) 131–137.
12 Kemp, J.A. and Sillito, A.M., The nature of the excitatory transmitter mediating X and Y cell inputs to the cat dorsal lateral geniculate nucleus, J. Physiol. (Lond.), 323 (1982) 377–391.
13 Leresche, N., Jassik-Gerschenfeld, D., Haby, M., Soltesz, I. and Crunelli, V., Pacemaker-like and other types of spontaneous membrane potential oscillations of thalamocortical cells, Neurosci. Lett., 113 (1990) 72–77.
14 Marsden, C.A., Measurement of Transmitter Release, Wiley, London, 1984.
15 Moruzzi, G., The sleep-waking cycle, Ergebn. Physiol., 64 (1972) 1–165.
16 Salt, T.E., Mediation of thalamic sensory input by both NMDA receptors and non-NMDA receptors, Nature (Lond.), 322 (1986) 263–265.
17 Sillito, A.M., Murphy, P.C. and Salt, T.E., The contribution of the non-NMDA group of excitatory amino acid receptors to retinogeniculate transmission in the cat, Neuroscience, 34 (1990) 273–280.
18 Soltesz, I., Haby, M., Jassik-Gerschenfeld, D., Leresche, N. and Crunelli, V., NMDA and non-NMDA receptors mediate both high and low frequency synaptic potentials in the rat lateral geniculate nucleus, Soc. Neurosci. Abstr., 15 (1989) 516.19.
19 Steriade, M., Deschenes, M., Domich, L. and Mulle, C., Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami, J. Neurophysiol., 54 (1985) 1473–1497.
20 Steriade, M. and Llinas, R., The functional state of the thalamus and the associated neuronal interplay, Physiol. Rev., 68 (1988) 649–742.
21 Stutzmann, J.M., Lucas, M., Blanchard, J.C. and Laduron, P.M., Riluzole, a glutamate antagonist, enhances slow wave and REM sleep in rats, Neurosci. Lett., 88 (1988) 195–200.
22 Thomson, A.M., West, D.C. and Lodge, D., An N-methyl-aspartate receptor mediated synapse in rat cerebral cortex: a site of action for ketamine?, Nature (Lond.), 313 (1985) 479–481.
23 Watkins, J.C. and Olverman, H.J., Agonists and antagonists for excitatory amino acid receptors, Trends Neurosci., 10 (1987) 267–272.
24 Wong, B.Y., Coulter, D.A., Choi, D.W. and Prince, D.A., Dextrorphan and dextromethorphan, common antitussives, are antiepileptic and antagonize N-methyl-D-aspartate in brain slices, Neurosci. Lett., 85 (1988) 261–266.