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Local perfusion of the thalamus with GABA increases sleep and induces long-lasting inhibition of somatosensory event-related potentials in cats

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The extracellular concentration of γ-aminobutyric acid (GABA) was increased in the ventroposterolateral nucleus of the thalamus in cats using in vivo microdialysis probes. In freely moving cats, the permanent injection of 8×10^{-9} M/mm^2 × min GABA induced a significant increase in sleep proportion. The duration of paradoxical sleep was particularly increased resembling the effects of benzodiazepines. In chloralose anesthesia, a similar increase in GABA concentration in the thalamus induced a tonic decrease in the peak-to-peak amplitude of cortical event-related potentials evoked by stimulation of the radial nerve. Following 10–15 min of inhibition during which the responses were as small as 20% of the original ones, the potentials started to recover. Finally, the responses were stabilized at a reduced amplitude. The present data suggests the important role of the thalamic GABAergic neurons in the regulation of sleep.

The involvement of GABAergic transmission in sleep regulation has been studied by the application of benzodiazepines. The interaction between benzodiazepine and γ-aminobutyric acid (GABA) receptors is known to enhance the efficiency of GABAergic transmission [2] which in turn induces a decrease in wakefulness duration and an increase in sleep duration [9]. However, the involvement of GABAergic interneurons of a well-defined brain area in the regulation of sleep has not been analysed. Recently, the contribution of thalamic GABAergic transmission to the EEG synchronizing mechanisms has been studied on thalamic slices [12]. Intracellular recordings from thalamic relay neurons suggest that the thalamic transmission of the sensory information is mainly controlled by GABAergic interneurons [3, 5, 12] by switching the bursting and stochastic firing modes of the relay cells in correlation with the alteration of sleep and wakefulness [6, 12].

A local increase in the GABAergic transmission could be achieved by the application of GABA into a small area of the brain due to the high-affinity GABA uptake.

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The in vivo microdialysis technique allows the permanent perfusion of a specified brain area with GABA without injection of a detectable volume of fluid into the brain. The transport of GABA from the dialysis fiber can be calibrated [6]. The effect of the injected GABA remains local due to the small size of the sampler. In the present study, the effects of a sustained increase of the extracellular GABA concentration in the ventro-posterolateral thalamic nuclei (VPL) on sleep and on the transfer of somatosensory information were investigated.

The measurements were carried out on adult cats. Four of them were anesthetized by Nembutal (50 mg/kg, i.p.) and electrodes were implanted into the skull for polygraphic recording of sleep stages. Dialysis probes were implanted into the ventro-posterolateral thalamic nuclei (A: 9, L: 7, V: −1). The probes were made of Travenol dialysis fibres (200 μm, o.d.) inserting two glass capillaries into them. 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 ferrule type tube connector cemented to the skull. The transport properties and the dead volume of samplers were measured in vitro by an electrochemical microcell system as described previously [6]. Adding 5×10^{-3} M GABA to the perfusion solution, the outflow was 10% in vitro. Consequently, the transported amount of GABA into the extracellular space of VPL was 8×10^{-9} M/mm^2 × min at 2 μl/min flow rate which was not an extremely high dosage compared to its uptake.

Following implantation, the dialysis probes were perfused by artificial cerebrospinal fluid (ACSF: pH 7.3; Na, 140 mEq; K, 3 mEq; Mg, 1 mEq) for 4 h each day. The perfusion fluid contained 10^{-6} M cytosine-arabinoside to reduce the development of a glial barrier around the sampler [6]. The sleep stages were differentiated by the usual polygraphic criteria and the recording was performed for 4 h each day from 09.00 to 13.00 h. The application of 5×10^{-3} M GABA in the perfusion solution was alternated by control sessions for 10 consecutive days. For the statistical evaluation of the sleep pattern the relative durations of sleep stages were compared by Student's t-test. The position of the sampler was checked by histological evaluation of Nissl-stained sections.

The effect of GABA on thalamic transmission of somatosensory evoked potentials was studied in 6 cats anesthetized by Chloralose (80 mg/kg, i.p.). A bipolar stimulating electrode was implanted to the left radial nerve. The skull was exposed above the representation of the radial nerve in the contralateral primary somatosensory cortex (SI) and a Ag/AgCl electrode of 150 μm in diameter was placed to the cortical surface. A dialysis probe was implanted into the right VPL at the same coordinates as in the chronic preparations. The stimulation of the radial nerve was performed by rectangular pulses of 0.2 ms duration with 0.1 Hz frequency. The stimulus amplitude was adjusted to the threshold of the motor response of the forelimb. Averaging 20 responses the results were plotted out. The relative peak amplitudes of the primary positive and negative components of the responses were measured using data of the first 10 averaged responses as 100%. The dialysis probe implanted into the VPL was perfused by ACSF for 1 h. The flow rate was 2 μl/min. Then the perfusion fluid was changed to another one containing 5×10^{-3} M GABA. The recording of the aver-

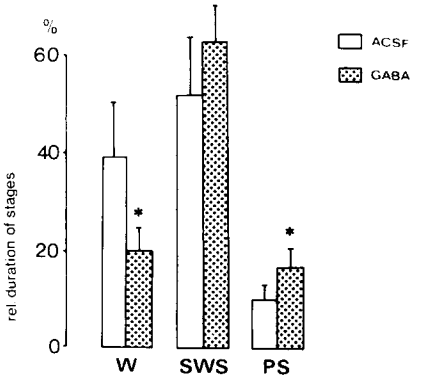

Fig. 1. Percentage of wakefulness (W), slow-wave-sleep (SWS) and paradoxical sleep (PS) applying artificial cerebrospinal fluid (ACSF) and ACSF plus γ-aminobutyric acid (GABA) in the perfusion solution through the dialysis probe implanted to the ventroposterolateral thalamic nuclei. *Changes which were found to be significant.

aged evoked responses was performed continuously for 2.5 h. To check the spontaneous changes in the responses, a 2.5 h long perfusion with ACSF containing no GABA was applied in 3 cats.

In the chronically implanted cats, the continuous and localized injection of GABA into the extracellular space of the VPL by microdialysis significantly decreased the time of wakefulness (W) ($P < 0.001$). The average ratio of slow-wave-sleep (SWS) to paradoxical sleep (PS) was also changed from 5.17 to 3.74 (Fig. 1) which was mainly due to the increase of PS. The increase of SWS was not significant (Fig. 1). No additional behavioral effect was observed as a consequence of GABA perfusion.

The evoked potentials recorded in SI were highly reproducable in the control periods. The relative variance was 8.1% of the average amplitude of the primary positive wave and 14.5% of the primary negative one. The peak amplitudes were not changed during 2.5 h when the VPL was perfused with ACSF (Fig. 2A). Application of 5×10^{-3} M GABA in the perfusion solution decreased the primary positive and negative components of the evoked responses (Fig. 2A). The maximum of the inhibition appeared 4–5 min following the onset of GABA perfusion when the peak amplitude of the primary positive wave was about 20% of the control value (Fig. 2A). The GABA-induced decrease of evoked responses was accompanied by a change in the pattern of the response. The decrease in the primary positive components was followed by the complete disappearance of the negative one. The restoration of the evoked response started with an increase in the primary negative wave (Fig. 2B). A decrease in the late positive component was also observed (Fig. 2B). Following 12–15 min of inhibition, the evoked responses started to increase again but they failed to reach the original amplitude. The original pattern of the responses also recovered (Fig. 2B). No changes, resembling the effects of GABA were found in cats where only ACSF was perfused (Fig. 2A).

The local application of GABA in the VPL, similarly to the effects of benzodiazepines, induced increase in the total amount of sleep [9]. We observed an increase in

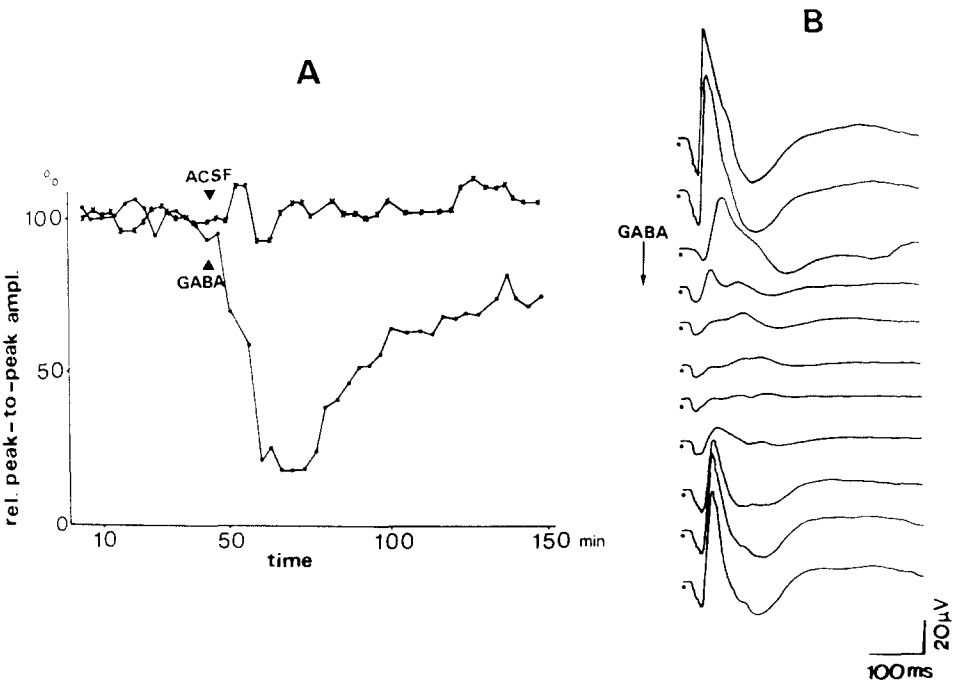

Fig. 2. Changes of the primary positive component of the somatosensory evoked potentials in SI following application of γ-aminobutyric acid (GABA) in the dialysis probe. A: the relative changes of the peak amplitudes of the primary positive responses using the average amplitude of the first 10 averaged responses as 100%, × marks the control data, ● marks the effects of GABA. ↓ indicates the start of GABA injection. B: the effect of GABA on the shape of the somatosensory evoked responses, ● indicate the stimulation. Twenty responses were averaged.

PS following long-term injection of GABA; application of midazolam in freely moving cats has been reported to produce the same effect [9]. This finding suggests that the benzodiazepine-induced increase in sleep might be due to the thalamic enhancement of GABAergic transmission.

The local increase in the extracellular GABA pool by microdialysis is likely to increase the GABAergic transmission because the freshly taken up GABA is known to get released preferentially from the nerve ending [10]. The astrocytes are probably unable to dump it because 80% of the extracellular GABA excess is taken up by the fast, neuronal GABA uptake system [11].

The enhanced inhibitory influence of GABAergic interneurons in the thalamus has been suggested to be responsible for the change of the functional state of the thalamic relay cells [7, 12] which is accompanied by a decrease in the information transmission [1, 4, 12]. Since it is known that the inhibition of the sensory inputs could induce sleep, it may be assumed that the modification of the sensory information transmission is an important component of the mechanism of sleep induction by increased GABAergic transmission. Our present findings appear to support this assumption. The partial recovery of the somatosensory evoked responses in spite of the perma-

nent injection of GABA into the extracellular space of VPL could be the consequence of the induction of a high-affinity GABA uptake system [10, 11] or it could be due to the receptor desensitization [8].

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