Brainstem Acetylcholine Release and REM Sleep

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SUMMARY: Many recent studies indicate that acetylcholine (ACh) in the brainstem is involved in REM sleep generation. Local ACh release in the brainstem regions, responsible for REM sleep during sleep and wakefulness, has not so far been reported because of technical difficulties. With in vivo microdialysis and electrochemical detection, we investigated the relationship between ACh release and sleep-wake stages in the dorsolateral pohs, the nucleus magnocellularis(NMC) and the nucleus paramedianus of the medulla of cats, which are indispensable for REM sleep generation, and the nucleus caudatus, as a control region. There were two patterns of ACh release in relation to sleep-wake stages; ACh release was significantly highest during REM sleep, or else it was not significantly different among sleep-wake stages'(wakefulness, slow-wave sleep and REM sleep). The REM sleep-specific enhancement of ACh release was observed in the dorsolateral pons and NMC, thus indicating the involvement of REM sleep-related cholinergic mechanisms in these regions. In the current paper, we report further studies of the pontine and medullary regions implicated in REM control with the microdialysis technique. We found that the medullary glutamate receptive neurons modulate both ACh release in the pons and REM sleep duration. Glutamate application within the pons produced an increase followed by a decrease in ACh release. These results indicate that interactions between glutamatergic and cholinergic cells in NMC and dorsolateral pons are important in REM sleep control.

KEY WORDS: Acetylcholine, Glutamate, Dorsal pons, Medulla, *In vivo* microdialysis, REM sleep.

Rapid eye movement (REM) sleep discovered by Aserinsky and Kleitman[l] is a unique stage of sleep characterized by cortical EEG desynchronization, absence of activities in the antigravity muscles (atonia), periodic burst of rapid eye movement, hippocampal rhythmic theta waves and ponto-geniculo-occipital(PGO) spikes. We would like to briefly review studies on the mechanisms underlying REM sleep generation and present our recent experiments.

Where is REM sleep generated?

Transection and lesion studies

when transections are placed at the junction of the midbrain and the pons, all the brainstem signs specific for REM sleep are observed caudal to the cut [2, 3, 4]. Transection between the spinal cord and the medulla (encephale isole) does not prevent all the signs of REM sleep from occurring rostral to the cut [5, 6]. From the above-mentioned studies, the structures caudal to the midbrain and rostral to the spinal cord seem to be necessary for REM sleep generation. Siegel *et al.* [7,8,9] made more detailed determination of REM sleep generating areas. These transection studies indicate that the pon$ is the critical area for the generation of much of the phenomenology of REM sleep.

Lesion studies were done in order to localize further the REM generating centres [10,11]. These results showed that the lateral region of the nucleus reticularis pontis oralis, ventral to the locus ceruleus, is most critical for REM sleep generation.

Unit recording studies

Unit recordings are important for clarifying the neuron groups involved in REM sleep generation. The lateral pontine reticular formation [12], and the medial- medulla [12,13,14] contain a population of cells that discharges at a high rate exclusively during REM sleep (REM-on cell).

Anatomical and pharmacological studies

It has been found that there exist many small-sized cholinoceptive neurons and cholinergic terminals in the dorsolateral pontine area [15,16]. A recent work reported that REM-on cells in the lateral region of pons are not cholinergic but cholinocfeptive [17]. Carbachol injections into the dorsal pontine area induce REM sleep with short latency [18, 19]. Studies in the decerebrated cat have demonstrated that muscle atonia could be induced by microinjection of glutamate as well as carbachol into the pontine muscle tone inhibitory region [20]. The atonia inducing effectsofacetylcholine(ACh)andglutamate have been found to be mediated by NMDA and M2 receptors, respectively [20,21 j.

In summary, REM sleep generating mechanisms have been Localized to cholinoceptive non cholinergic cells in the dorsolateral pons.

In Vivo measurement of local neurotransmitter release in relation to sleep-wake cycles

As mentioned above, there are cholinoceptive REM-on neurons and cholinergic terminals in the dorsolateral pons, and cholinergic agonists induce REM sleep at short latency wheffmicroinjected in this region. If REM-on cells in the dorsolateral pons are normally involved in the executive system of REM sleep, there should be a specific increase of ACh release at cholinergic terminals during or prior to REM sleep. However, it was very difficult to measure the local release ^transmitters associated with REM sleep in such a small brain region.

In the early experiments the cortical cup method [22] and a push-pull cannulation method [23] were used to measure ACh release at the cortical surface of cats. Gadea-Ciria *et al* [24] measured ACh release in the caudate nucleus of cats during sleep and wakefulness with a push-pull cannula and a radioenzyme assay. However, the brain regions where they measured ACh are not directly related to REM sleep generation and push-pull cannulation causes substantial damage to the brain tissues around the tip of the cannulae.

The brain dialysis method was developed as an alternative method of push-pull cannulation, and later refined to a microdialysis method with the use of semi-permeable membrane [25].

Principle of microdialysis The microdialysis method uses the principle of dialysis. The semipermeable membrane separates brain interstitial fluid from the m perfusing solution. If there is a difference in the concentra tion of any substance to which the membrane is permeable, the concentration gradient causes diffusion of the substance from interstitial space into dialysis membrane. (When the substance is added to perfusion liquid in higher concentration than in the interstitial fluid, the substance diffuses out of die membrane and into the tissuearound the membrane). **^

Advantages of microdialysis method are: (1) Since the perfusing liquid does not contact directly to the tissue, the damage to the tissue is relatively small compared to a push-pull cannulation method. This allows more prolonged collection of samples. (2) As samples can be collected serially from freely moving animals without disturbing sleep, we can investigate a relationship between the release of neurotransmitters and animal behaviours.

Precaution during use of microdialysis for sleep studj^i Perfusion rate, temperature of perfusate, dialysis-tube length, composition of perfusate (da^{2}*, pH etc.) time after probe insertion, draining-effeet are factors that affect t substance collection. There are three criteria to keep ia mind when we measure neuronal release of a substance from the terminals; TTX(tetrodotoxin) sensitivity of tite change, Ca * dependency and an increase of release produced by high K^{+} concentration Ringer solution are the necessary conditions to determine whether the release is neurogenic or not [26,27,28}. The inserted probe damages the tissue, and prolonged insertion of a probe will cause the change of the microenvironment by tissue gliosis. We have found that the most reliable results are obtained if samples are collected for 24 hours, starting at least 12 hrs after insertion.

Together with the improvement of the system for neurotransmitters, such as high perfo liquid chromatography with electro-chemical the present microdialysis method has enabled us measure various neurotransmitters in a small quantity, *vivo* and *in situ.* Measurement of ACh by the iSicrodiarysa^ method under pharmacological stimulation has reported since 1987 [29,30,31]. Recently, the number of such brain neurotransmitter studies has been increasing. However, there are only few reports on the relationship between endogenous brain neurotransmitters sleep-wake stages, because Hie physiological changes ii their concentration during the sleep-wake cycle art quite small compared to those produced by electrical and pharmacological stimulation.

Acetylcholine

We, for the first time, measured local brainstem ACh release in relation to sleep-wake stages [32]. ACh release was measured both in the dorsolateral pontine area, a possible REM-generating locus, and in the caudate nucleus, which is a subcortical region rich in ACh but not directly related to the REM-generation system, in cats. ACh release during REM sleep in the dorsolateral pons was about two times higher ($p < 0.001$) than those during slow-wave sleep (SWS) and wakefulness (Table 1). ACh release was consistently highest during REM sleep in each session and each animal. On the other hand, ACh release in the caudate nucleus was not significantly different between REM sleep and Wakefulness. ACh release in the caudate was significantly higher during REM sleep and wakefulness than during SWS (Table 1). The overall mean amount of ACh release was 10 times higher in the caudate nucleus than in the dorsolateral pons. These results indicate that a REM sleep-specific enhancement of ACh release is noted in the dorsolateral pons, a REM generating centre, bat not in the caudate nucleus. Our previous studies in decerebrated animals have shown that cholinergic stimulation, but not glutamate stimulation of the caudal portion of medulla, corresponding to the nucleus paramcdianus (NPM), triggers atonia [20]. In contrast,

Table 1. Acetyicholine and glutamate release across sleep-waking cycle in cats brain(mean ± S.E.). (FTD: dorso-lateral area of the pons; NMC: nucleus magnocellularis of the medulla; NPM: nucleus paramedianus of the medulla; CA: caudate nucleus.)

Acetycholine (f mol/ min.)

	no. of sample	SWS	REM	WAKE
FID	15	27.72 * 1.75	51.67 * 1.92	32.23*1.35
NMC	12	49.67 * 3.13	55.78 ± 2.87	54.83 * 3.67
NPM	12	24.22*1.25	31.66 * 1.75	23.45*1.64
CA	15	232,0 ±*542*	313.6 * 74.0	300.8 * 73.6

Glutamate (% release, SWS = 100)

		SWS	REM	WAKE
FID	15	100	97.9 * 4.6	100.5 * 3.6
NMC	15	100	107.0 ±6.0	93.2*6.1 .
NPM	15	100	98.9 ± 4.9	92.3 * 5.5

glutamate, but not cholinergic, stimulation is effective in triggering muscle atonia in the rostral area, corresponding to the nucleus magnocellularis (NMC). In NPM and NMC, we measured ACh release during sleep-waking monitoring [33]. As shown in Table 1, ACh release in NPM is significantly higher during REM sleep than during SWS and wakefulness. By contrast, ACh release in NMC was not significantly different between REM sleep and wake-fulness. ACh release in NMC was lowest during SWS. These results indicate that the cholinoceptive neurons in NPM are related to REM sleep, especially muscle atonia. The number of microdialysis studies dealing with changes in ACh release related to sleep-wake stages and circadian rhythm are now gradually increasing. Lydic et al. reported an increase of ACh release in the dorsal pons of cats during carbachol-induced REM sleep-like state [34}. Kametani and Kawamura reported changes in ACh release across sleep-wake cycle in the hippocampus, the cerebral cortex and the caudate nucleus of rats [35, 36]. Their results indicated that ACh release was higher during wakefulness and REM sleep than during SWS. Because of the absence of REM sleep-specific increase of ACh release, these three areas may not be directly involved in REM sleep generation.

Glutamate

Our group found the evidence that glutamate may be involved in REM sleep control. Glutamate injection into the dorsal pons and NMC induced muscle atonia *in* decerebrated cats [20]. We measured glutamate release in three areas, the dorsolateral pons, NMC and NPM(Tablc 1). The change in glutamate release across sleep-wakefulness was not large compared with that in ACh release. We found that glutamate release in NMC was slightly higher during REM sleep than in waking and slow-wave sleep. However this cbangewas not significant.

Others

Many neuronal transmitters and neuropeptides, such as dopamine [25], norepinephrine [37], serotonin (5 HT) [38], amino acids [39] and adenosine [40], have been measured with microdialysis technique for the past decade. However, there are few reports of neurotransmitters related to sleep or circadian rhythms. Histamine release has a circadian rhythm in the hypothalamus of freely moving rats [41]. Circadian changes in 5HT [42], norepinephrin^[43] and melatonin [44] release have also been reported. Mori's group reported that there was a decrease of 5HT release during REM periods in cats' pontine reticular formation(personal communication). A sleep specific change of GABA in the pontine area was observed in our preliminary study.

What regulates acetylcholine release in the pons?

There is a REM sleep-specific increase of ACh release in the dorsal pons. The next question is which part of the brain is responsible for the change in ACh release in the dorsal pons. There are two possible ways to affect pontine ACh

release. One is a change in activity of cholinergic neurons in or projecting to the pons from the medulla or forebrain. Another one is the interaction between ACh and another neurotransmsitters. For example, glutamate might affect ACh release as it has been reported to affect dopamine release [45].

Extrinsic input

Although there are reports that the basal forebrain and the hypothalamus affect the REM sleep, transection studies indicate that the inputs from these areas are not required for basic REM phenomena. However, the interaction between the medulla and the pons is thought to be important [8]. Sakai *et al.* reported that there are cholinergicprojections from NMCto the pons [46] and that these projections are necessary for REM sleep. Therefore, we examined the effect of electrical or chemical stimulations of NMC on ACh release in the pons.

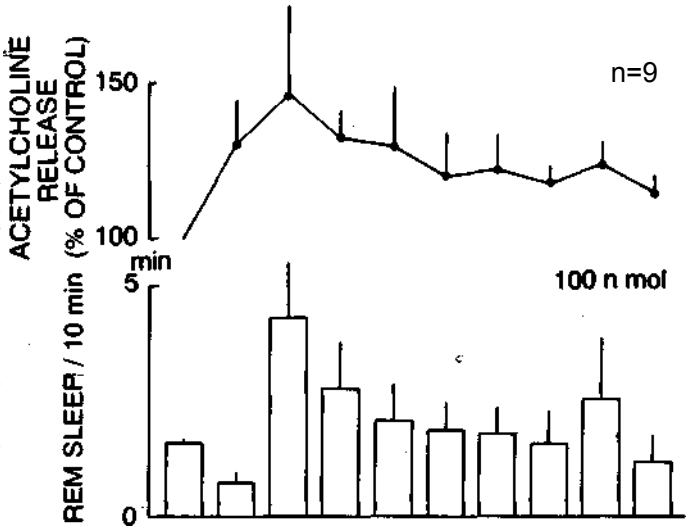

fig 1. Change in acetyleholine release and REM sleep amount after glutamate microinjection (100 n mol/0.5 uJ) into NMC(n«9). Upper: ACh release measured every 10 min after glutamate stimulation. Each data point is shown as a mean of a % release of prestimulation value. ACh release increased until the second period and then decreased gradually. Lower: REM sleep amount (per 10 min) after glutamate stimulation. REM sleep amount changes correlated with ACh release.

We placed a microdialysis probe into the dorsolateral pons. We activated NMC with electrical stimulation, carbachol injection or glutamate injection. Electrical stimulation with 30 sec trains of 0.1 msec pulses at 20 Hz significantly enhanced ACh release in the dorsolateral pons during the first, third and fifty 5 min periods after, stimulation. This stimulation significantly shortened REM latency to 19.1 min in comparison to 33.0 min in the control, but did hot significantly affect the amount of REM sleep during one hour after stimulation [32].

Microinjection of glutamate (100 nmol/0.5 \L\ ) into NMC during wakefulness enhanced ACh release(Fig. 1). This microinjection significantly shortened REM latency to 10.1 min and also significantly enhanced the amount of REM during one hour to 16.1 min compared with 9.6 min in the control. In contrast, microinjection of carbachol (40 nmol/0.4 \n\) into NMC during wakefulness failed to affect ACh release, REM latency and the amount of REM sleep. Injection of carbachol into NMC tended to arouse the cats. This is consistent with our finding that there is no significant increase of ACh release in NMC during REM sleep (Table 1). Therefore dialysis and stimulation data indicate that ACh release in NMC may not be important in REM sleep generation. On the other hand, though it is not significant, there is a slight increase of glutamate release during REM sleep in NMC as shown in Table 1. We have found that glutamate stimulation of this region produces atonia [20]. These results suggest that a non-cholinocep-tive, glutamate-receptive system in NMC is involved in the REM sleep-specific enhancement of ACh release in the dorsal pons.

Intrinsic modulation

We have found that glutamate injection into the dorsal pons triggers atonia. However, we could not detect a significant change in glutamate release in the dorsal pons during sleep-wake cycle (Table 1). The reason is not clear. But there are two possibilities. One is that synaptic release in the pons cannot be detected because it is overwhelmed by

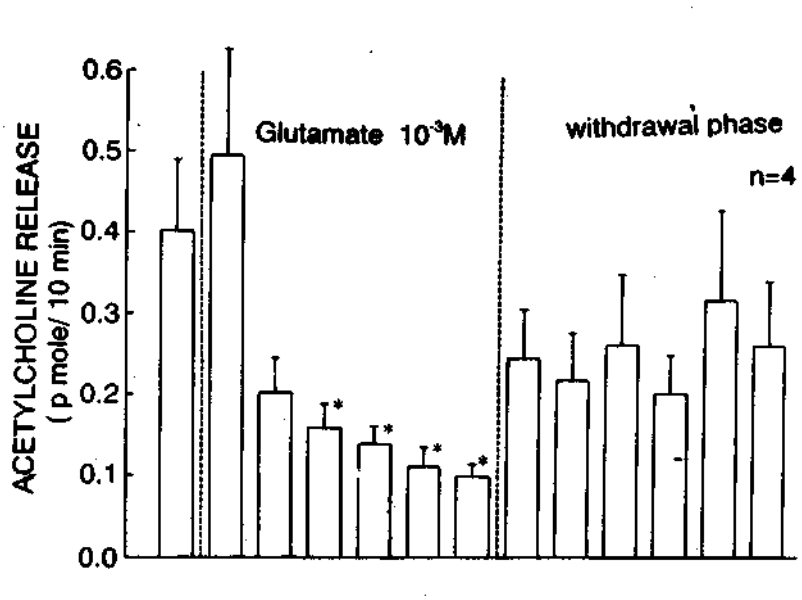

Fig. 2. Acetyldloline release measured at 10 min intervals during and after glutamate stimulation (10^{-3} M). During glutamate perfusion ACh release significantly decreased(asterisk). After withdrawal, it returned to the pre-stimulation level.

non-synaptic release. Another possibility is that it is not the specific increase of glutamate release during REM sleep but the glutamate-ACh interaction that is important for REM generation. We tried to stimulate the dorsolateral portion of pons with glutamate through microdialysis probe and simultaneously measured ACh release with the same probe. During the continuous glutamate stimulation, ACh release was increased initially, but later significantly decreased. ACh release then returned to the control level after glutamate stirnulation(Fig. 2). During glutamate stimulation, REM sleep amount was only 1.8 min/ 60 min. On the other hand, during the withdrawal phase, the REM amount was significantly increased to 12.2 min. The REM latency after stimulation was 15.3 min and significantly shorter than the control one.

It has been reported that the function of glutamate receptor is changed by ACh release [47]. Our data showed that ACh release was modulated by glutamate. Therefore, the interaction between ACh and glutamate could contribute to the REM generation system.

It is clear that cholinoceptive neurons in the dorso-lateral pontine reticular formation are important in generating REM sleep. There is evidence that several neurotransmitters and neuropeptides modulate ACh release. Clarification of the interaction between these substances is critical for an understanding of REM sleep control.

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