

## Enhancement of acetylcholine release during REM sleep in the caudomedial medulla as measured by in vivo microdialysis

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Previous studies in our laboratory have found that muscle atonia could be triggered by two distinct areas of the medial medulla, a caudal region, corresponding to the nucleus paramedianus (NPM) and a rostral region, corresponding to the nucleus magnocellularis (NMC). The former region is responsive to acetylcholine (ACh) and the latter region is responsive to glutamate. In this study we have measured the endogenous ACh release across the sleep–wake cycle in these two areas with the microdialysis technique in unanesthetized, freely moving cats. We found that ACh release in NPM was state-dependent and was about 30% higher ( $P < 0.001$ ) during rapid eye movement (REM) sleep than during slow-wave sleep and wakefulness. However, ACh release in NMC was not selectively elevated in REM sleep. The enhancement of ACh release in NPM during REM sleep supports our hypothesis that ACh release onto cholinergic neurons in this area mediates the muscle atonia of REM sleep.

Muscle atonia, the tonic feature characteristic of rapid eye movement (REM) sleep<sup>5,17</sup>, can be induced by two supraspinal structures, the pontine reticular formation and the medial medulla<sup>14</sup>. A loss of muscle tone can be evoked by injection of the cholinergic agonist carbachol into the pontine inhibitory area (PIA), which corresponds to dorsolateral regions of the nucleus reticularis pontis oralis<sup>17</sup>. Recent studies show that there are many small-sized cholinergic neurons and cholinergic terminals in PIA<sup>4,7</sup>. A specific increase of ACh release is also found in this region prior to and during REM sleep<sup>6</sup>. The second area in which stimulation can elicit atonia is the medial medulla<sup>3,12</sup>. Our previous studies in decerebrate animals have shown that glutamate stimulation but not ACh stimulation of the rostral portion of the medial medulla, corresponding to the nucleus magnocellularis (NMC), will trigger atonia<sup>8</sup>. In contrast, cholinergic stimulation but not glutamatergic stimulation is effective in the caudal portions of this area, corresponding to the nucleus paramedianus (NPM)<sup>8</sup>. However, there are cholinergic neurons both in NMC and NPM<sup>1,15</sup>. If cholinergic neurons in NPM are responsible for muscle atonia, there should be a specific increase of ACh release in this area during REM sleep. In the present study, we attempted to clarify the relationship between local ACh release in NPM and NMC and sleep stages in cats using the microdialysis technique.

Four adult cats (two males and two females) weighing 3.0–4.5 kg were anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg i.p.) and chronically implanted with standard sleep electrodes for recording cortical electroencephalograms (EEG), ponto-geniculo-occipital (PGO) waves, eye-movement potentials (EOG) and nuchal electromyograms (EMG). Guide cannulae (21G) for microdialysis probes were also stereotaxically implanted above the target regions, NMC (A –9.5, L 1.0 D –7.5 to –9.5) and NPM (A –13.5, L 1.0, D –7.0 to –9.0) (Fig. 1). After the experiment, the positions of the probes were histologically verified.

Following recovery from surgery, at least 12 h prior to the collection of dialysate, a microdialysis probe was inserted, through the guide cannulae into NMC or NPM. Brain microdialysis and sleep monitoring were simultaneously performed between 08.00 h and 17.00 h in the freely moving, unanesthetized cat. A coaxial type of microdialysis probe, made of polyamido-coated quartz glass, was covered by a semipermeable membrane at its tip (O.D.: 220  $\mu$ m; length: 2 mm; 90% cut off, 50 kDa; supplied by EICOM, Kyoto. Ringer solution containing 0.1 mM physostigmine, a specific acetylcholinesterase inhibitor, was perfused at the flow rate of 2  $\mu$ l/min. Dialysate was collected for 5 min during a specific sleep–waking state and injected into the mobile phase. The mobile phase was 100 mM phosphate buffer (pH 8.5), contain-

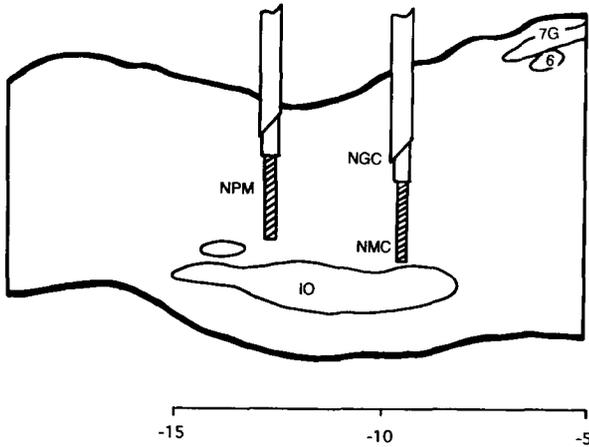


Fig. 1. A diagram demonstrating the positions of microdialysis probes. The tip (semipermeable membrane) of the probe is located in NPM (A -13.5, L 1.0, D -7.0 to -9.0) and in NMC (A -9.5, L 1.0, D -7.5 to -9.5). The shaded portions indicate semipermeable membrane. NPM, nucleus paramedianus of medulla; NGC, nucleus gigantocellularis; IO, inferior olive; 6, nucleus abducens; 7G, genu of the facial nerve.

ing 0.6 mM tetramethylammonium and 0.55 mM 1-decanesulfonic acid (NAKARAI, sodium salt). ACh level in the perfusate was determined by a high-performance liquid chromatography (HPLC) and electrochemical detection (ECD) system (EICOM). The coefficient of variation in the determination of 1 pmol of acetylcholine and choline by this HPLC-ECD system was 1.4% and 2.6%, respectively. ACh peak height on the ECD recorder was linearly correlated with the amount of ACh applied to the HPLC-ECD system within the range between 0.06 and 5.0 pmol.

Twelve dialysate samples were obtained during each stage of wakefulness (W), slow-wave sleep (SWS) and REM sleep from both NMC and NPM. A two-way analysis of variance (sleep-wake stage by region) with replicated measures ( $n = 12$ ) and paired  $t$ -tests revealed that the amount of ACh release was significantly ( $P < 0.001$ ) different among W, SWS and REM and also significantly ( $P < 0.001$ ) different between NMC and NPM. The relationship of ACh release to sleep-wake stages was different in NMC and NPM (interaction,  $P < 0.05$ ). In NPM (Fig. 2A), the mean ( $\pm$  S.E.) amount of ACh release during REM sleep ( $31.66 \pm 1.75$  fmol/min perfusion) was 31% higher than that during SWS ( $24.22 \pm 1.25$  fmol) and 35% higher than that during W ( $23.45 \pm 1.64$  fmol). These differences were both significant ( $P < 0.001$ ). In NMC (Fig. 2B), by contrast, the mean amount of ACh release was not significantly different between REM ( $55.78 \pm 2.87$  fmol) and W ( $54.83 \pm 3.67$  fmol). ACh release in NMC during REM or W was significantly higher ( $P < 0.01$ ) than that during SWS ( $49.67 \pm 3.13$  fmol).

These results indicate that the pattern of ACh release is different in NMC and NPM. ACh release is significantly higher during REM than during SWS and W in

NPM, but not in NMC. It is noteworthy that this increased release is observed only in the region where ACh microinjection induces muscle atonia.

The pattern of ACh release in NPM and NMC can be compared with that in PIA. The ACh release in PIA during REM sleep ( $51.67 \pm 1.92$  fmol/min dialysate, 15 cases) is markedly higher than that during SWS ( $27.72 \pm 1.75$  fmol) and W ( $32.23 \pm 1.35$  fmol). This pattern is similar to that in NPM but not to that in NMC. Furthermore, the microinjection of ACh and/or ACh analogs into NPM induces muscle atonia<sup>8</sup>. These findings are consistent with the hypothesis that cholinergic neurons in NPM are responsible for muscle atonia, but, that cholinergic neurons in NMC are not.

In contrast to NPM, ACh is not selectively released in NMC in REM sleep. The level of ACh release in NMC during REM sleep is almost the same as that during W and both W and REM sleep rates are higher than that during SWS. There are a considerable number of putative cholinergic neurons firing highly both in REM and W<sup>2,13,18</sup>. The elevated ACh release increase which we see both in REM sleep and waking may be related

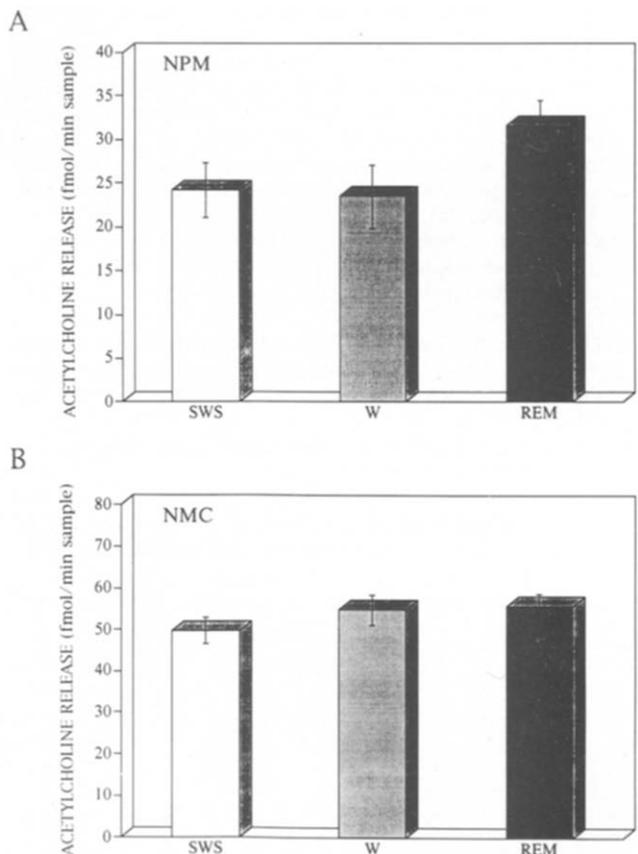


Fig. 2. Mean and standard error of acetylcholine (ACh) release during SWS, REM sleep and W in A, the nucleus paramedianus of medulla (NPM) and B, nucleus magnocellularis (NMC). Ordinate, the amount of ACh release (fmol per 1 min sampling time).

to the activities of this neuronal type. The average basal ACh release in NMC is higher than that in NPM. This suggests a difference in the tonic level of cholinergic neuron terminal activation, but it is difficult to assess the significance of differences in the absolute levels of ACh in the dialysate<sup>10</sup>. The tonic release of ACh in NMC may be related to cardiovascular control, since microinjections of cholinergic agonists in this area have a potent effect on blood pressure and heart rate<sup>9</sup>.

ACh release in PIA and NPM is high during REM sleep and relatively low both during SWS and W. ACh release in these two regions is likely to be related to the activities of ACh neurons active during REM sleep, but relatively inactive in W. Cholinergic regions in PIA and NPM receive projections from dorsolateral pontine cholinergic neurons<sup>11,16</sup>.

We hypothesize that muscle atonia generated by cholinergic neurons is mediated by two distinct pathways. One pathway is via glutamatergic and cholinergic neurons in pons projecting to glutamate sensitive, non-cholinergic neurons in NMC. These NMC neurons then project to the spinal cord to either activate interneurons responsible for muscle atonia, or directly hyperpolarize motoneurons. The other pathway is via cholinergic neurons in the pons projecting to cholinergic neurons in NPM which in turn project to the spinal cord. The increase of ACh release in NPM during REM sleep that we find in the present study supports the existence of a REM-sleep-active cholinergic pathway to NPM.

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