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SHORT COMMUNICATION

Effects of dexmedetomidine, midazolam, and propofol on acetylcholine release in the rat cerebral cortex in vivo

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Abstract Acetylcholine plays an important role as a neurotransmitter in the central nervous system with involvement in both sleep and arousal. Dexmedetomidine, midazolam, and propofol are widely used for sedation of patients in intensive care medicine. In this study, we have examined the effect of continuous administration of dexmedetomidine, midazolam, and propofol on acetylcholine release in the rat cerebral cortex, using an in vivo microdialysis technique. Following infusion of a control solution, male Wistar rats ($n = 6$/group) were administered dexmedetomidine at 0.3 μg/kg/min, midazolam at 20 mg/kg/h, or propofol at 50 mg/kg/h over a 2-h period. Using a brain microdialysis method, extracellular acetylcholine concentrations were measured up to 2 h after administration of each agent at 15-min intervals. In the midazolam group, acetylcholine levels were significantly reduced with midazolam infusion, remaining low even after the drug was stopped. In the propofol group, acetylcholine levels were significantly decreased during propofol infusion, but returned to control levels once the infusion was stopped. Dexmedetomidine administration decreased acetylcholine release, but this finding was not statistically significant. From this study, midazolam and propofol but not dexmedetomidine significantly suppressed acetylcholine release in the cerebral cortex at sedative doses. Even though the righting reflex recovered almost the same after the cessation of drug administration, midazolam suppressed acetylcholine release longer than propofol.

Keywords Acetylcholine · Microdialysis · Neurotransmitter · Sedation

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Various types of drugs are used for the sedation and analgesia of patients in anesthesia and intensive care medicine. Dexmedetomidine is known to exert its sedative and analgesic effects through α_2-adrenergic receptors [1, 2], acting via the locus coeruleus for its sedative effect and via α_2-adrenergic receptors in the dorsal horn of the spinal cord for its analgesic effect [3]. Midazolam and propofol, which act on GABA receptors [4–8], were the predominant analgesic drugs used before dexmedetomidine.

Acetylcholine is a cerebral neurotransmitter that has been shown to play roles in sleep, arousal, memory, and attention [9, 10]. Cholinergic neurons are widely distributed in the cerebral cortex and are involved in the control of sleep and wakefulness [11, 12].

Previous microdialysis studies have shown that propofol, a GABA receptor agonist, suppresses acetylcholine release in the cerebral cortex [13]. However, the effects of α_2-receptor agonist dexmedetomidine and the GABA receptor agonist midazolam on acetylcholine release in this region are not well known. To clarify the effect of these agents on acetylcholine release from the cerebral cortex, we examined acetylcholine release in the cerebral cortex using brain microdialysis. Furthermore, we determined whether the sedative state can account for cholinergic suppression alone.

A total of 18 male Wistar rats (180–250 g) were used. All experiments were started between 7:00 and 9:00 a.m. Each rat was anesthetized by the intraperitoneal administration of pentobarbital (50 mg/kg), and a dummy cannula

was inserted into the right frontal cortex (3 mm lateral, 3 mm anterior, and 2 mm ventral to the bregma) according to Paxonos and Watson [14]. A 24-G intravenous cannula was inserted through the caudal vein. The rats were allowed to recover for at least 24 h after surgery. On the day of the experiment, the dummy cannula was removed, a straight-type dialysis probe (A-I-8-03; Eicom, Kyoto, Japan; 3-mm-long dialysis membrane) was inserted, and microdialysis was performed. The rat was placed in a clear plastic box and allowed to move freely throughout the study. The dialysis probe was perfused with Ringer's solution supplemented with 2×10^{-4} M eserine at a rate of 2 μl/min using a perfusion pump (ESP-32; Eicom) with a 2.5-ml gas-tight syringe. During dialysis, the perfusate and an internal standard solution (0.025 M phosphate buffer, pH 3.5, containing 5×10^{-7} M isopropyl homocholine and 10 mM EDTA-2Na) were automatically pumped into the HPLC system at 15-min intervals. The HPLC-based acetylcholine assay was started 120 min after the start of perfusion, and samples were analyzed at 15-min intervals. Rats were divided into three treatment groups before measurements were begun: (1) dexmedetomidine ($n = 6$), (2) midazolam ($n = 6$), and (3) propofol ($n = 6$). As a baseline, four samples were collected in the first 60 min after 120 min of perfusion and subjected to measurement. Dexmedetomidine, midazolam, or propofol were then infused through the caudal vein at rates of 0.3 μg/kg/min, 20 mg/kg/h, or 50 mg/kg/h, respectively, over 2 h using a microinfusion pump. The doses of intravenous anesthetics used were determined from a previous study [15, 16] and preliminary experiments. Following infusion, measurements were continued for a further 2 h. After the injection of each agent we evaluated the righting reflex. Samples were separated on a polymer-based reverse-phase column (4.6 mm × 150 mm; EICOMPAK AC-GEL; Eicom), subjected to enzymatic reaction on an enzyme column (AC-ENZYMPAK; Eicom), and then quantified by electrochemical detection (ECD300; Eicom). The mobile phase consisted of 10 mM $KHCO_3$, 150 mg/l decane sodium sulfonate, and 50 mg/l EDTA-2Na, and was delivered with a pump system (EP-300; Eicom) at a rate of 0.6 ml/min. Measurements were performed at 33 °C in a column temperature control unit (ACT-300; Eicom). Values are presented as percentages relative to basal acetylcholine release. Basal acetylcholine release was calculated as the mean of the four samples obtained before the infusion of each agent. Results are expressed as the mean ± standard deviation (SD). Statistical analyses were performed by analysis of variance (ANOVA) and unpaired *t* test with Bonferroni's correction. Differences between groups were tested by a two-way ANOVA followed by Tukey's post hoc tests. Differences were considered significant at $p < 0.05$.

Following dexmedetomidine administration, the activity of the rats decreased immediately and righting reflexes were lost within 15 min. Following dexmedetomidine withdrawal, righting reflexes recovered within 15 min. Rat activities also recovered within 15 min to approximately the same levels observed before infusion. Acetylcholine release was suppressed by dexmedetomidine infusion, with a maximum suppression of 74 % ± 12 % of the baseline level (Fig. 1). However, this was not statistically significant.

Following midazolam administration, rat activities decreased immediately and righting reflexes were lost within 15 min. After midazolam withdrawal, righting reflexes recovered within 15–30 min, but rat activities were reduced compared with that before infusion. Significant suppression of acetylcholine release was observed after 45 min of midazolam infusion. Acetylcholine release reached a plateau, and thereafter, release was suppressed to 43 % ± 13 % of the baseline level. Even after midazolam withdrawal, acetylcholine release remained at 40–50 % of baseline levels.

Following propofol administration, the activity of the rats decreased immediately and righting reflexes were lost within 15 min. After propofol withdrawal, righting reflexes and rat activities both recovered within 15 min to the same levels observed before infusion. Significant suppression of acetylcholine release was observed after 45 min of propofol infusion. Propofol infusion maximally suppressed acetylcholine release to 32 % ± 16 % of the baseline level. After the withdrawal of propofol infusion, acetylcholine release increased and no significant difference was observed compared with the control 30 min after

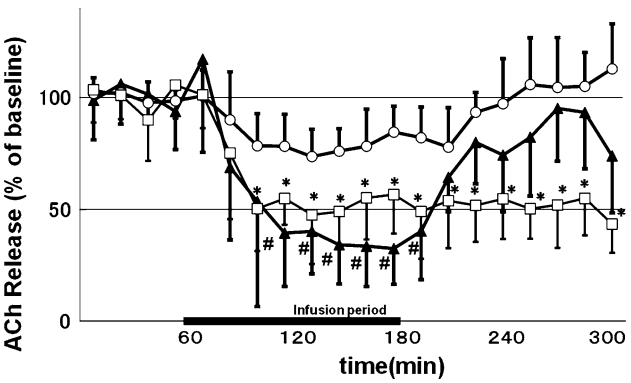

Fig. 1 Effect of dexmedetomidine, midazolam, and propofol on acetylcholine (Ach) release from the rat cerebral cortex. Acetylcholine release was significantly suppressed 45 min after propofol and midazolam infusion. Withdrawal of propofol, but not midazolam, was followed by an increase in acetylcholine release. There was no significant difference in acetylcholine release compared with baseline in the dexmedetomidine group. $^{\#,*}p < 0.05$ versus baseline value; *filled triangles* propofol; *open circles* dexmedetomidine; *open squares* midazolam

discontinuation. Moreover, propofol and midazolam also significantly suppressed acetylcholine release compared with dexmedetomidine 30 min after administration. In addition, 30 min after discontinuation of the drugs, midazolam significantly suppressed acetylcholine release compared with dexmedetomidine and propofol (Fig. 2a, b).

Our study demonstrates that acetylcholine release in the cerebral cortex declines during the sedative state induced by dexmedetomidine administration, although these effects were not statistically significant. A study examining the effect of dexmedetomidine on acetylcholine release in the thalamus found no relationship between dexmedetomidine and acetylcholine release [17]. Electroencephalogram and immunohistochemical studies have shown that the sedative state evoked by dexmedetomidine is similar to non-REM sleep observed during natural sleep [18, 19]. Acetylcholine release from the cerebral cortex is suppressed during the state of natural sleep [9]. In this study, although loss of the righting reflex was observed in the dexmedetomidine group, acetylcholine release was not significantly suppressed at the sedative dose. A previous study [16] used a larger dose of dexmedetomidine to induce loss of the righting reflex; however, we used minimal drug doses to evoke loss of righting reflex similar to the other two drugs from preliminary experiments. The depth of sedation evaluated from the righting reflex was almost the same for all drugs. Therefore, we concluded that the depth of the sedation was sufficient to enable comparison of the drugs.

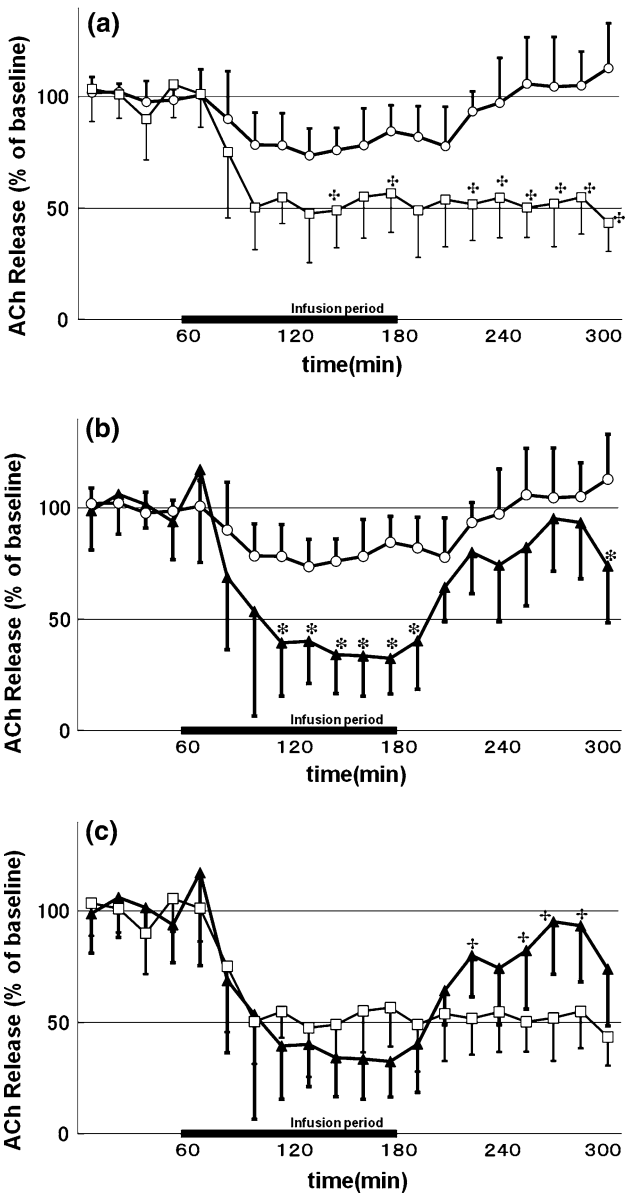

Fig. 2 **a–c** Acetylcholine (Ach) levels were compared between each group. ✣,❋,✣$p < 0.05$; *filled triangles* propofol; *open circles* dexmedetomidine; *open squares* midazolam

Moreover, our study found that the GABAergic activators midazolam and propofol both significantly suppressed acetylcholine release in the cerebral cortex. These findings are consistent with a previous study showing that a single intraperitoneal dose of propofol suppressed acetylcholine release in the cerebral cortex [13]. However, the mechanism by which GABA agonists interact with cholinergic neurons remains undetermined. Our results show that the sedative state induced by midazolam and propofol, and potentially other GABA agonists as well, may cause suppression of acetylcholine release in the cerebral cortex.

The relationship between the suppression of acetylcholine and the depth of sedation is unknown. A previous study reported that the recovery periods from loss of the righting reflex were not different between midazolam and propofol [20]. Another study investigating the intravenous administration of midazolam demonstrated that spontaneous locomotor activity was strongly suppressed even after the righting reflex was recovered [21] Comparing the drugs, propofol and midazolam significantly suppressed acetylcholine compared with dexmedetomidine, despite similar losses of the righting reflex. After discontinuation of the drugs, only midazolam continued to suppress acetylcholine release compared with propofol and dexmedetomidine although the righting reflex was recovered (Figs. 1, 2a,c). The sustained suppression caused by midazolam after its discontinuation suggests that the sedative effects may persist even after the patient is awake. Therefore, observation of the patients for several hours after the emergence from anesthetics may be beneficial.

In conclusion, midazolam suppressed acetylcholine release in the cerebral cortex, and this suppression was sustained even after the discontinuation of midazolam infusion. Furthermore, midazolam-induced suppression of acetylcholine did not coincide with loss of the righting reflex. Acetylcholine release was strongly suppressed immediately following propofol infusion, but returned to

control levels once the infusion was stopped. Acetylcholine release was suppressed by dexmedetomidine infusion, but not significantly. Therefore, the involvement of acetylcholine in the sedative state and its recovery is different for each of the drugs investigated. For this reason, the sedative state cannot account for cholinergic suppression alone. Moreover, loss of the righting reflex could also not explain the suppression or inhibition of the acetylcholine in the cerebral cortex. Further studies are needed to clarify the mechanisms underlying the sedative state.

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