In vivo effects of propofol on acetylcholine release from the frontal cortex, hippocampus and striatum studied by intracerebral microdialysis in freely moving rats

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Summary
Using in vivo microdialysis, we have investigated the effects of propofol on acetylcholine (ACh) release from various regions of the rat brain. Propofol 25 and 50 mg kg\(^{-1}\) i.p. decreased basal ACh release from the frontal cortex by 70% and 85%, respectively. Propofol 25 and 50 mg kg\(^{-1}\) i.p. decreased basal ACh release from the hippocampus by 47% and 72%, respectively. However, in rat striatum, propofol 25 mg kg\(^{-1}\) i.p. did not affect basal ACh release and 50 mg kg\(^{-1}\) i.p. produced slight inhibition of basal ACh release (by 19%) only in the second sample after i.p. injection. In addition, we also examined the pharmacological mechanisms mediating the interaction between propofol and a γ-amino-butyric acid A (GABA\(_A\)) receptor complex. In the rat hippocampus, local application of bicuculline 1 μmol litre\(^{-1}\), a GABA\(_A\) receptor antagonist, significantly antagonized the inhibitory effects of propofol 50 mg kg\(^{-1}\) i.p. on basal ACh release. In the rat frontal cortex, local application of bicuculline 1 μmol litre\(^{-1}\) did not antagonize the inhibitory effects of propofol 50 mg kg\(^{-1}\) i.p. on basal ACh release, while systemic application of bicuculline 1 mg kg\(^{-1}\) i.p. significantly antagonized the inhibitory effects of propofol 50 mg kg\(^{-1}\) i.p. These results suggest that propofol has powerful depressant effects on ACh release from the rat frontal cortex and hippocampus but not from the striatum, indicating that propofol has a “region-selective” anaesthetic action. Further, these results suggest that the inhibitory effects of propofol in the rat hippocampus may involve “intra” hippocampal GABA\(_A\) receptors while the inhibitory effects in the rat frontal cortex may be mediated by GABA\(_A\) receptors other than “intra” frontal cortex GABA\(_A\) receptors. (Br. J. Anaesth. 1998; 80: 644–648)

Keywords: anaesthetics i.v. propofol; parasympathetic nervous system acetylcholine; measurement techniques microdialysis; brain cerebral cortex; brain hippocampus; rat

The effects of anaesthetic agents are complex: they produce loss of consciousness, loss of memory, changes in spontaneous activity, attenuation of protective reflexes, loss of postural reflexes and unfavourable side effects such as hallucinations, euphoria and amnesia. In addition, anaesthetic agents may influence the level or homeostasis of neurotransmitters in the brain, such as dopamine, norepinephrine and acetylcholine (ACh).\(^1\)\(^2\) ACh was the first neurotransmitter to be described and cholinergic neurones are distributed widely in the brain.\(^3\)\(^4\) Using in vivo microdialysis, several studies reported that anaesthetic agents affect ACh release in various brain regions.\(^5\)\(^6\)\(^7\) Cholinergic mechanisms are known to be important in the striatum, where a balance between dopamine and ACh release ensures normal motor output,\(^4\) and in the hippocampus and frontal cortex, where ACh has long been known to play a key role in the regulation of consciousness, learning and memory.\(^5\) As ACh may be one of the major neurotransmitters affecting the action of anaesthetics, the pharmacological study of cholinergic mechanisms in anaesthesia is important.

Propofol (2,6-disopropylphenol), a short-acting i.v. general anaesthetic, has been in clinical use since the 1980s and has been used widely for induction and maintenance of anaesthesia because of its versatility and favourable pharmacokinetics.\(^8\) Propofol is believed to potentiate the inhibitory actions of the γ-amino-butyric acid A (GABA\(_A\)) receptor and its pharmacological mechanisms have been investigated in some detail. However, the molecular mechanisms by which the hypnotic effects of propofol occur are not fully understood.\(^9\)\(^10\)

In this study, we examined the effects of propofol on ACh release from the rat frontal cortex, hippocampus and striatum in freely moving rats, and investigated the pharmacological mechanisms mediating the interaction of propofol with the GABA\(_A\) receptor, using a brain microdialysis technique, coupled with a highly sensitive analytical method based on high performance liquid chromatography with electrochemical detection (HPLC-ECD).

Materials and methods
The study was approved by the Animal Welfare Committee of Yokohama City University School of Medicine. We studied adult male Sprague–Dawley rats (weighing 250–350 g). The animals were housed in cages at room temperature (22°C), maintained on a 12-h light–dark cycle (lights on 07:00–19:00 h) and were given free access to water and food. The animals
were anaesthetized with pentobarbital 40 mg kg\(^{-1}\) i.p. and placed in a stereotaxic apparatus (Model SR-6, Narisige Scient Instrument LAB., Japan). The skull was exposed and a guide cannula (CMA 10, Carnegie Medicine, Sweden) for penetration of a microdialysis probe was stereotaxically implanted into the right frontal cortex (coordinates taken from the bregma with the skull flat: A: 3.3 mm, L: 2.8 mm, V: 0.5 mm from the atlas of Paxinos and Watson\(^{11}\)), the right hippocampus (coordinates from the bregma: A: -5.6 mm, L: 5.0 mm, V: 3.8 mm) and the right striatum (coordinates from the bregma: A: 1.0 mm, L: 3.0 mm, V: 4.0 mm), fixed with dental cement and fastened with four screws onto the skull of the rat. Microdialysis combined with HPLC-ECD in freely moving rats was performed, essentially as described previously,\(^3\)\(^4\) at least 2 days after implantation of the guide cannula to allow for surgical recovery. The probe (CMA 11, Carnegie Medicine, Sweden) (2-mm long dialysis membrane for the right frontal cortex and 3-mm long dialysis membrane for the right hippocampus or striatum, od 0.24 mm, molecular weight cut-off = 20 000 Da) was inserted into the guide cannula under diethyl ether anaesthesia on the day of the microdialysis experiment.

Rats were allowed to move freely in a bowl-shaped cage. During the experiments, the microdialysis probes were perfused with Ringer’s solution containing eserine sulphate 10 \(\mu\)mol litre\(^{-1}\) (Wako Pure Chemical Industries Ltd, Japan), an inhibitor of acetylcholine esterase (AChE), at a rate of 2.0 \(\mu\)l min\(^{-1}\) controlled by a micro infusion syringe pump (Model CMA 102, Carnegie Medicine, Sweden). Rats were pre-perfused with Ringer’s solution for 120 min, and then every 20 min perfusate was collected in a chilled polyethylene tube containing 10 \(\mu\)l of ethylhomo-choline 1 mmol litre\(^{-1}\), an internal standard for HPLC determination.

The test drugs used were propofol (Zeneca Pharma., Japan) 25 and 50 mg kg\(^{-1}\) i.p. and (−)-bicuculline methylchloride (RBI, Natick, MA, USA), a GABA\(_A\) receptor antagonist, 1 \(\mu\)mol litre\(^{-1}\) for local application or 1 \(\mu\)g kg\(^{-1}\) i.p. for systemic application. A new rat was used for each drug dose tested.

In our preliminary investigations, we confirmed the anaesthetic effects of propofol 10, 25 and 50 mg kg\(^{-1}\) i.p. in adult male Sprague–Dawley rats. Propofol 25 and 50 mg kg\(^{-1}\) produced loss of righting reflex and anaesthetic state within approximately 5–10 min after i.p. administration. A state of anaesthesia continued for approximately 60–90 min. A dose of propofol 10 mg kg\(^{-1}\) did not produce observable changes in behaviour in all rats. Therefore, we used doses of 25 and 50 mg kg\(^{-1}\) i.p. The i.p. route was favourable for experiments using freely moving rats because of ease of administration and reliability.\(^{12,13}\)

After collecting three basal samples, propofol was administered i.p. Bicuculline 1 \(\mu\)mol litre\(^{-1}\) was dissolved in perfusate and given locally via the microdialysis probe from the start of experiments (local application), or bicuculline 1 \(\mu\)g kg\(^{-1}\) was given i.p. 20 min before propofol i.p. administration (systemic application). ACh release was measured by HPLC-ECD. Polymeric reversed-phase column (Eicom-pak AC-GEL, 6.0 × 150 mm, Eicom, Japan) was used for HPLC-ECD separation. An AC-Enzympak column (ACh esterase and choline oxidase-immobilized,
Eicom, Japan) was used to convert ACh and choline to hydrogen peroxides, which were detected by an electrode (Model ECD-100, Eicom, Japan) at 450 mV. The mobile phase was phosphate buffer 100 mmol litre\(^{-1}\) (pH 8.5), containing tetramethylammonium chloride 65 mg litre\(^{-1}\) and decansulphonic acid sodium salt 200 mg litre\(^{-1}\). The limit of sensitivity, defined as a signal-to-noise ratio >4, was 100 fmol (100 fmol/50 \(\mu\)l injection) for ACh. At the end of the experiments, the rats were killed using an overdose of diethyl ether, and the position of the probe in the brain was confirmed by visual examination. ACh recovery from the microdialysis probes was measured at the end of the experiment. In vitro ACh recovery (performed at 37 °C) through 3-mm and 2-mm long dialysis membranes at a rate of 2 \(\mu\)l min\(^{-1}\) was mean 14.5 (SEM 0.5) % \((n=15)\) and 11.0 (0.5) % \((n=10)\), respectively.

Results are expressed as (mean (SEM)) percentage of basal ACh release. Basal release was obtained from the mean of three initial collections before administration of the test drugs. The significance of differences between mean values was determined by ANOVA with repeated measures, followed by Scheffe’s F test for multiple comparisons. \(P<0.05\) was accepted as significant.

Table 1: Peak effects of systemic (−)-bicuculline (BICUC) 1 and 10 \(\mu\)g kg\(^{-1}\) i.p. and propofol 50 mg kg\(^{-1}\) i.p., alone or in combination, on ACh release from the rat frontal cortex. BICUC was given i.p. 20 min before propofol. Values are mean (SEM) percentage of baseline. \(*P<0.05\) compared with bicuculline 1 \(\mu\)g kg\(^{-1}\); \(†P<0.05\) compared with propofol 50 mg kg\(^{-1}\) i.p. alone (ANOVA followed by Scheffe’s F test for multiple comparisons).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Peak effects (% of baseline)</th>
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<tbody>
<tr>
<td>BICUC alone</td>
<td>105.3 (11.8)</td>
</tr>
<tr>
<td>10 (\mu)g kg(^{-1}) i.p. ((n=5))</td>
<td>152.7 (22.6)*</td>
</tr>
<tr>
<td>Propofol 50 mg kg(^{-1}) i.p. ((n=5))</td>
<td>15.8 (3.1)*</td>
</tr>
<tr>
<td>Propofol 50 mg kg(^{-1}) i.p. +</td>
<td>50.4 (13.6)*†</td>
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Figure 4: A: Effects of propofol 50 mg kg\(^{-1}\) i.p. \((n=5)\) and bicuculline (BICUC) 1 \(\mu\)mol litre\(^{-1}\) (local perfusion) alone \((n=5)\) or in combination \((n=5)\), on ACh release from the rat frontal cortex. B: Effects of propofol 50 mg kg\(^{-1}\) i.p. \((n=5)\) and bicuculline (BICUC) 1 \(\mu\)mol litre\(^{-1}\) alone \((n=5)\) or in combination \((n=5)\), on ACh release from the rat hippocampus. \(*P<0.05\) compared with bicuculline alone; \(†P<0.05\) compared with propofol 50 mg kg\(^{-1}\) i.p. alone.

Results

ACh release was stable over 120 min after commencing the perfusion. Baseline concentration of ACh in the frontal cortex, hippocampus and striatum in 20-min samples were 0.9 (0.1) pmol sample\(^{-1}\) \((n=15)\), 1.8 (0.3) pmol sample\(^{-1}\) \((n=15)\) and 14.8 (1.0) pmol sample\(^{-1}\) \((n=18)\), respectively.

Figure 1 shows the effects of propofol on ACh release from the rat frontal cortex. Propofol 25 and 50 mg kg\(^{-1}\) i.p. decreased basal ACh release from the frontal cortex. Mean maximal decreases produced by propofol 25 and 50 mg kg\(^{-1}\) i.p. were 69% and 85%, respectively. Peak values of the mean decrease were measured in the second sample after i.p. injection (No. 5 fraction).

Figure 2 shows the effects of propofol on ACh release from the rat hippocampus. Propofol 25 and 50 mg kg\(^{-1}\) i.p. decreased ACh release from the hippocampus. Mean maximal decreases produced by propofol 25 and 50 mg kg\(^{-1}\) i.p. were 51% and 72% of basal levels, respectively. Peak values of the mean decrease were measured in the second sample after i.p. injection (No. 5 fraction).

Figure 3 shows the effects of propofol on ACh release from the rat striatum. In the rat striatum, propofol 25 mg kg\(^{-1}\) i.p. did not affect ACh release and 50 mg kg\(^{-1}\) i.p. produced slight inhibition of ACh release (19%).

In the hippocampus, local application of bicuculline 1 \(\mu\)mol litre\(^{-1}\) significantly antagonized the inhibitory effect of propofol 50 mg kg\(^{-1}\) i.p. (by 57.4%), while bicuculline 1 \(\mu\)mol litre\(^{-1}\) alone failed to alter ACh release (fig. 4n). However, in the rat frontal cortex, local application of bicuculline 1 \(\mu\)mol litre\(^{-1}\) did not significantly antagonize the inhibitory effect of propofol 50 mg kg\(^{-1}\) i.p. on ACh release (fig. 4a).

Table 1 shows the effect of systemic application of GABA\(_A\) antagonist on ACh release from the frontal cortex. Although bicuculline 10 \(\mu\)g kg\(^{-1}\) i.p. increased ACh release to 152% of basal values in the frontal
cortex, bicuculline 1 μg kg−1 i.p. failed to alter ACh release. Systemic pretreatment of bicuculline 1 μg kg−1 i.p. significantly antagonized the inhibitory effect of propofol 50 mg kg−1 i.p. (by 50.4%), while bicuculline 1 μg kg−1 i.p. alone failed to alter ACh release.

Discussion

Brain microdialysis provides in vivo information on a variety of neurotransmitters in the extracellular space of the brain. In our microdialysis study, we investigated the effects of propofol on ACh release from various regions of the rat brain, including the frontal cortex, hippocampus and striatum.

Many reports have shown that general anaesthetics affect a variety of neurotransmitters in various rat brain regions. In our previous study, we reported the inhibitory effects of pentobarbital on ACh release from the frontal cortex, hippocampus and striatum, which may imply that pentobarbital is a powerful depressant anaesthetic in all brain regions. Our present data showed that propofol decreased ACh release much more effectively in the frontal cortex or hippocampus than in the striatum, indicating that propofol has a “more selective” anaesthetic action than other depressant anaesthetics such as pentobarbital.

It is generally accepted that ACh in the striatum is an important neurotransmitter in the extrapyramidal motor neurone, regulating locomotion and movement. ACh in the hippocampus may be involved in cognitive functions such as memory and learning while ACh in the frontal cortex is concerned with concentration, attention span, initiative and spontaneity.

Propofol has gained widespread use because of the quality and speed of recovery. The magnitude and duration of impairment of psychomotor performance after propofol anaesthesia is distinctly less than that after barbiturate anaesthesia. The results of our present study that propofol produced smaller effects on striatal ACh release compared with pentobarbital may explain the more rapid recovery of psychomotor function compared with barbiturate anaesthesia.

Propofol is chemically unrelated to all other anaesthetic agents, such as barbiturates or benzodiazepines. Furthermore, the precise binding site of propofol is unknown, although it may be different from that of barbiturates or benzodiazepines. It has been proposed that propofol could act by binding directly to the GABA<sub>A</sub> chloride channel or to the channel regulatory proteins. Propofol has been shown to have a potent effect on GABA-activated chloride channels in rat brain and large effects on chloride conductance in Xenopus oocytes expressing mouse brain in mRNA. The mechanism by which propofol potentiates GABA-mediated transmission is unlikely to involve inhibition of GABA reuptake, because the actions of muscimol and 3-aminopropanesulfonic acid (two structural analogues of GABA that are poor substrates for the GABA uptake systems) were also potentiated by propofol. Bicuculline antagonizes GABA<sub>A</sub> receptors by competing with GABA for its binding sites and is known to induce convulsions. Schulz and Macdonald reported that barbiturates directly activate bicuculline-sensitive chloride conductance.

As shown in figure 4A, our data demonstrated that propofol produced inhibitory effects on ACh release via “intra” hippocampal GABA<sub>A</sub> receptor mechanisms, at least in part in the rat hippocampus, which strongly supports the hypothesis that there may be a relationship between propofol and GABA<sub>A</sub> receptor mechanisms. However, as shown in figure 4A, local application of bicuculline did not significantly antagonize the inhibitory effects of propofol 25 and 50 mg kg<sup>−1</sup> i.p. on ACh release in the rat frontal cortex. In contrast, systemic application of bicuculline 1 μg kg<sup>−1</sup> i.p. (no effective dose) significantly antagonized the inhibitory effects of propofol 50 mg kg<sup>−1</sup> i.p., as shown in table 1. Furthermore, the finding that systemic application of bicuculline 10 μg kg<sup>−1</sup> i.p. alone increased ACh release from the frontal cortex indicates that ACh release from this region may be tonically inhibited by GABA<sub>A</sub> receptor activity. The reason why there is no effective action of locally applied bicuculline in the rat frontal cortex is unknown. One possibility is that “extra” frontal cortex GABA<sub>A</sub> receptor mechanisms may be involved in the inhibitory effects induced by propofol on ACh release from the rat frontal cortex. Further investigations are needed to elucidate the precise cellular mechanisms.

Studies of pentobarbital in primates have shown that it depresses metabolism in all brain areas. On the other hand, in the clinical setting, using positron emission tomography, the effects of propofol on cerebral glucose metabolic rates showed that propofol did not uniformly depress metabolism throughout all brain regions. Metabolism in the cortex was depressed more than that in subcortical regions, indicating that some regions were affected more than others. These findings are comparable with ours; propofol produced significant inhibition of ACh release in the frontal cortex and hippocampus but only slight inhibition in the striatum in vivo. Therefore, propofol appears to have “more selective” anaesthetic actions than barbiturates. However, the precise pharmacological mechanisms and binding site of propofol are unknown.

In summary, propofol had a powerful depressant action on ACh release in the rat frontal cortex and hippocampus but not in the striatum, suggesting that propofol has a “locality selective” anaesthetic action. Furthermore, the inhibitory effects of propofol may be mediated by “extra” frontal cortex GABA<sub>A</sub> receptors in the frontal cortex, and “intra” hippocampal GABA<sub>A</sub> receptors in the hippocampus.

References


