SHORT COMMUNICATION

Chronic ethanol consumption does not affect action of propofol on rat hippocampal acetylcholine release in vivo


Department of Anesthesiology, Yokohama City University School of Medicine, 3–9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

*Corresponding author. E-mail: inagawa@med.yokohama-cu.ac.jp

Background. The aim of this study was to examine ethanol-consumption-related changes in the effects of propofol on rat hippocampal acetylcholine (ACh) release.

Methods. Male Sprague–Dawley rats received a solution of ethanol (20% v/v) for 24 weeks while controls received tap water. The effects of propofol were examined by in vivo microdialysis, with ACh release from the hippocampal regions determined by high-performance liquid chromatography with electrochemical detection (HPLC–ECD).

Results. Propofol 50 mg kg\(^{-1}\) i.p. significantly decreased basal hippocampal ACh release in ethanol-treated and control rats by 50.4 (SEM 4.7)% and 38.3 (11.1)% respectively. Propofol 100 mg kg\(^{-1}\) i.p. significantly decreased basal hippocampal ACh release in ethanol-treated and control rats by 67.5 (3.7)% and 55.9 (7.4)% respectively. The reduction in hippocampal ACh release induced by 50 or 100 mg kg\(^{-1}\) i.p. propofol was not significantly different between ethanol-treated and control rats. There was no significant difference in the duration of sleep between the two groups.

Conclusions. These results demonstrate that chronic ethanol consumption does not augment the inhibitory actions of propofol on rat hippocampal ACh release. These findings appear to be inconsistent with the notion that chronic ethanol intake enhances the propofol-induced inhibition of the hippocampal cholinergic system and related mental dysfunction.

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enhances or prolongs the inhibitory actions of propofol on rat hippocampal ACh release, as does ageing, and that changes in anaesthetic modulation of hippocampal ACh release related to ethanol consumption may underlie postoperative cognitive impairment in patients who are alcohol abusers.

Methods and results
The study was approved by the Animal Care and Use Committee at Yokohama City University School of Medicine. Following the methods of previous studies, male Sprague–Dawley rats were administered ethanol in drinking water (20% v/v) while control animals received tap water. The ethanol concentration was initially 5%, and was increased progressively by 5% every week until a 20% concentration was reached. This was then maintained for 24 weeks. After the treatment period, ethanol was withdrawn. Subsequently, animals were maintained on water for 4 weeks before experiments were started to avoid the possible effects of withdrawal. Experimental animals did not show the typical signs of ethanol dependence, such as tremors of the tail and rigidity of the tail or body.

The microdialysis technique combined with high-performance liquid chromatography with electrochemical detection (HPLC–ECD) in freely moving rats was performed as previously described. A guide cannula for penetration of the microdialysis probe was implanted into the right hippocampal region under pentobarbital anaesthesia. At least 2 days elapsed before the measurements. After 120 min of dialysis equilibration, the dialysate was collected every 20 min. After collecting three initial samples, propofol (Zeneca Pharma, Japan) was administered intraperitoneally. An anaesthetic state was defined as the LRR, and we evaluated the duration of LRR. ACh recovery through the probe at a flow rate of 2 μl min⁻¹ was 14.6 (SEM 1.2)% (n=8).

All results are expressed as means (SEM). Values are expressed as a percentage of basal ACh release, with basal release being the average of the three initial collections before administration of propofol. The time-dependent change in ACh release was analysed by ANOVA with repeated measures, followed by Fisher’s protected least significant difference test for multiple comparisons. The difference between the data obtained from the two groups was analysed using Student’s unpaired t-test. P<0.05 was regarded as significant.

Body weights in ethanol-treated and control rats were 552.0 (16.5) g and 613.0 (13.7) g, respectively. There was a significant difference between the groups. The duration of LRR with propofol 100 mg kg⁻¹ in ethanol-treated and control rats was 48.8 (5.6) min and 40.2 (15.7) min, respectively. There was no significant difference between the two groups. Propofol 50 mg kg⁻¹ i.p. did not induce LRR in either group.

ACh release was stable for 120 min after the beginning of perfusion. Baseline ACh release (with no correction for recovery) from the hippocampus in ethanol-treated rats and control rats, in 20-min samples, was 1.7 (0.2) pmol/20 min (n=12) and 2.2 (0.3) pmol/20 min (n=12), respectively. There was no significant difference between these values.

Propofol 50 mg kg⁻¹ i.p. significantly decreased basal hippocampal ACh release in ethanol-treated and control rats by 50.4 (4.7) % and 38.3 (11.1) %, respectively (Fig. 1A). Propofol 100 mg kg⁻¹ i.p. significantly decreased basal hippocampal ACh release in ethanol-treated and control rats by 67.5 (3.7)% and 55.9 (7.4)%, respectively (Fig. 1B). These changes induced by 50 or 100 mg kg⁻¹ i.p. propofol were not significantly different between ethanol-treated and control rats.

Discussion
In the present study, we administered ethanol in drinking water for 24 weeks and performed microdialysis after four ethanol-free weeks in order to eliminate the influences of
alcohol withdrawal and the persistence of alcohol in blood. Casamenti and colleagues demonstrated that the same paradigm of ethanol treatment resulted in a decrease in choline acetyltransferase with no change in basal ACh release in rat cerebral cortex and hippocampus. They also showed that the body weight in ethanol-treated rats was lower than that in control animals. Our findings for basal ACh release and body weight are consistent with their results, confirming that our ethanol intoxication model is reliable and comparable.

Chronic ethanol consumption is considered to cause hypofunction of the septohippocampal cholinergic system, although changes in basal hippocampal ACh levels vary depending on the model. We expected that the propofol-induced decrease in hippocampal ACh release might be prolonged in ethanol-treated rats, possibly due to impairment of the cholinergic nervous system, as observed in the ageing model. However, we did not find any significant difference in basal ACh release or the effects of propofol between ethanol-treated and control rats. In addition, there was no significant difference in the duration of LRR between the two groups after administration of the same dose of propofol corrected for body weight. These results suggest that chronic ethanol consumption and ageing differentially affect basal hippocampal ACh release, and that chronic alcohol intake does not enhance propofol-induced inhibition of the septohippocampal cholinergic system after a sufficient period for abstinence.

One of the pharmacological targets for ethanol is the GABA_A receptor, whose function and expression decrease after chronic administration of ethanol. It is known that septal GABAergic interneurones play an important role in controlling hippocampal ACh release. Our finding that there was no significant difference in the inhibitory effects of propofol on rat hippocampal ACh release between the two groups implies that GABA_A receptor function was preserved after a 24-week period of ethanol administration. Although the precise mechanisms have not been determined, one possible explanation is that impaired GABA_A receptor function in the hippocampus can recover during ethanol abstinence. Further studies are needed to clarify these suggestions.

In conclusion, our results demonstrate that there is no significant difference in basal ACh release or the inhibitory effects of propofol on rat hippocampal ACh release between ethanol-treated and control rats. These findings are inconsistent with the notion that chronic ethanol intake enhances propofol-induced inhibition of the hippocampal cholinergic system and related mental dysfunction.

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