Opposite effects of depressant and convulsant barbiturate stereoisomers on acetylcholine release from the rat hippocampus in vivo


Department of Anesthesiology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanasawa-Ku, Yokohama 236-0004, Japan

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

Background. It has been shown that the R(−) isomer of 1-methyl-5-phenyl-5-propyl barbituric acid (MPPB) induces loss of the righting reflex (LRR), while S(+)-MPPB causes pure excitatory effects, including convulsions, in vivo.

Methods. We studied the effects of the depressant and convulsant MPPB stereoisomers on rat hippocampal acetylcholine (ACh) release in vivo, using a brain microdialysis technique in freely moving animals.

Results. R(−)-MPPB 60 and 90 mg kg⁻¹ i.p. decreased ACh release from the rat hippocampus by 44.1 (8.2)% and 60.8 (8.2)%, respectively. In the hippocampus, the local application of bicuculline, a γ-aminobutyric acid (GABA)ₐ receptor antagonist, 1 μmol litre⁻¹ antagonized the inhibitory effects of R(−)-MPPB 90 mg kg⁻¹ i.p. In contrast, R(−)-MPPB, S(+)-MPPB 60 and 90 mg kg⁻¹ i.p. increased ACh release to 151.8 (6.8)% and 169.6 (11.1)% of the basal release, respectively.

Conclusions. Our results demonstrated that R(−)-MPPB decreased, while S(+)-MPPB increased, rat hippocampal ACh release and that the inhibitory effects of R(−)-MPPB may involve the GABAₐ receptor in vivo. These data imply that changes in hippocampal ACh due to these agents may be related to their central inhibitory and stimulatory actions in vivo.

Br J Anaesth 2004

Keywords: brain, acetylcholine; brain, microdialysis; isomer, R(−)-MPPB; isomer, S(+)-MPPB

Accepted for publication: September 24, 2003

It is known that barbiturates are optically active drugs and that some stereoisomers of barbiturates exhibit opposing, or less potent, pharmacological activities. Among these, the R(−) isomer of 1-methyl-5-phenyl-5-propyl barbituric acid (MPPB) induces loss of the righting reflex (LRR), whereas S(+)-MPPB causes pure excitatory effects, including convulsions, in vivo. These stereoisomers are useful to explore the mechanisms of barbiturate actions. We used these depressant and convulsant barbiturate stereoisomers to explore the potential roles of changes in brain ACh levels in the anaesthetic actions of barbiturates. If changes in the ACh level are related to the behavioural effects of the isomers, the finding would be consistent with the idea that modulation of ACh release by barbiturates is related to the depressive and excitatory actions on the CNS in vivo. In this study, we measured ACh release from the rat hippocampus in vivo, using brain microdialysis.

Methods and results

Hippocampal ACh levels were measured as previously described. Adult Sprague–Dawley rats (weighing 250–350 g), anaesthetized with sodium pentobarbital, were placed in a stereotaxic apparatus, and a guide cannula (CMA 10; Carnegie Medicine, Solna, Sweden) for the penetration of a microdialysis probe was implanted into the right hippocampal region (coordinates from bregma: A −5.6 mm, L 5.0 mm, V 3.8 mm). Microdialysis experiments were conducted under free-moving conditions at least 2 days after implantation of the guide cannula. A probe (CMA
11, Carnegie Medicine) (3 mm long dialysis membrane, O.D. 0.24 mm, MW cut-off = 20 000 Da) was perfused with Ringer solution containing 10 μmol litre⁻¹ eserine sulphate (Wako, Osaka, Japan), an inhibitor of ACh esterase, at a constant flow rate of 2.0 μl min⁻¹. After 120 min of equilibration, the dialysate was collected every 20 min into a chilled polyethylene tube containing 10 μl of ethylhumocline 1 μmol litre⁻¹, an internal standard for HPLC determination. After sampling three initial collections, the test drugs were administered i.p. The test drugs used for the experiment were R(−)-MPPB and S(+)-MPPB, kindly provided by Professor J. Knabe (Saarland University, Saarbrücken, Germany). The stereoisomers of MPPB were dissolved in 1 ml of dimethylsulphoxide (DMSO) 99%. (−)-Bicuculline methylchloride (RBI, Natick, MA, USA), a GABAₐ receptor antagonist, 1 μmol litre⁻¹ was dissolved in perfusate and given locally via the microdialysis probe from the start of experiments. Control rats were treated with the same volume of a vehicle. ACh was assayed by a method described previously.² The in vitro ACh recovery (carried out at 37°C) through a 3 mm long dialysis membrane at a speed of 2.0 μl min⁻¹ was 14.6 (1.2)% [mean (SEM), n=8].

All results are expressed as mean (SEM) and values are expressed as a percentage of basal ACh release. The basal release was obtained from the average of the three initial collections before administration of the test drugs. The significance of the differences among the corresponding samples obtained in different conditions was analysed by one-way ANOVA followed by Fisher’s protected least significant difference test for multiple comparisons. *P<0.05 was accepted as significant.

Baseline ACh release (with no correction for recovery) from the rat hippocampus in a 20 min sample was 2.2 (0.1) pmol/20 min (n=20). As shown in Fig. 1A, R(−)-MPPB 60 and 90 mg kg⁻¹ i.p. significantly decreased basal ACh release from the rat hippocampus by 44.1 (8.2) and 60.8 (8.2)%, respectively. Contrary to R(−)-MPPB, S(+)MPPB 60 and 90 mg kg⁻¹ i.p. increased ACh release to 151.8 (6.8) and 169.6 (11.1)% of the basal release, respectively (Fig. 1B). Local hippocampal application of bicuculline 1 μmol litre⁻¹ significantly antagonized the inhibitory effect of R(−)-MPPB 90 mg kg⁻¹ i.p., while local perfusion of bicuculline 1 μmol litre⁻¹ alone did not alter basal release from the hippocampus (Fig. 1C). In a separate group of animals (n=20, 5 for each condition), we confirmed that R(−)-MPPB 60 and 90 mg kg⁻¹ caused LRR and that S(+)MPPB at the same doses induced convulsions.

Comment

Our results demonstrated that the depressant R(−)-MPPB decreased, while the convulsant S(+)MPPB increased, rat

Fig 1 (A) Effects of R(−)-MPPB 60 mg kg⁻¹ (n=5) and 90 mg kg⁻¹ (n=5) i.p. on acetylcholine (ACh) release from the rat hippocampus. *P<0.05 compared with the vehicle in the corresponding samples; †P<0.05 for the comparison between 60 and 90 mg kg⁻¹ R(−)-MPPB i.p. (B) Effects of S(+)MPPB 60 mg kg⁻¹ (n=5) and 90 mg kg⁻¹ (n=5) i.p. on ACh release from the rat hippocampus. *P<0.05 compared with the vehicle in the corresponding samples; †P<0.05 for the comparison between 60 and 90 mg kg⁻¹ R(−)-MPPB i.p. (C) Changes in ACh release from the rat hippocampus were measured after R(−)-MPPB 90 mg kg⁻¹ i.p. injection in the presence (n=5) or absence (n=5) of local perfusion of bicuculline (BICUC) 1 μmol litre⁻¹. *P<0.05 compared with R(−)-MPPB i.p. and local perfusion of BICUC.
hippocampal ACh release in vivo, indicating opposite effects of MPPB isomers on hippocampal ACh release. The decrease in ACh release by R(−)-MPPB was partially antagonized by local application of bicuculline, a GABA_A receptor antagonist, indicating that depression of the hippocampal ACh levels by R(−)-MPPB is mediated by enhancement of the GABA_A function in the hippocampus, at least in part. These results, when combined with the effects on behaviour, imply that changes in hippocampal ACh levels caused by MPPB isomers may be related to the central depressive and stimulatory actions of these agents.

Central cholinergic neurons are distributed widely in the CNS and may be involved in unconsciousness induced in general anaesthesia. In rats, a decrease in the central ACh levels has been shown to increase anaesthetic potency of isoflurane and elevating ACh was shown to increase the anaesthetic requirement. Another study demonstrated that physostigmine, a cholinesterase-inhibitor, reversed propofol-induced unconsciousness in human volunteers. The reversal of unconsciousness was blocked by pretreatment with scopolamine. These findings suggest that central cholinergic transmission may play an important role during anaesthesia. The hippocampus receives abundant cholinergic extrinsic innervations from the medial septal area containing choline acetyltransferase-positive neurons. A recent study showed that inactivation of the medial septum or hippocampus by local injection of a GABA_A receptor agonist exaggerated the behavioural response to general anaesthetics, including the volatiles, pentobarbital and propofol, suggesting that the septohippocampal system participates in general anaesthesia. Because inactivation of the septohippocampal system probably leads to a decrease in hippocampal ACh release, it is likely that the modulation of hippocampal ACh release plays a role in anaesthetic action.

MPPB isomers are known to exhibit contrasting actions on GABA_A receptors, with stimulation by the depressant and inhibition by the convulsant. It is likely that the correlation between hippocampal ACh levels and behaviour mainly stems from the opposite effects on the GABA_A receptor function caused by the stereoisomers, as modulation of the GABA_A receptor function probably contributes to the changes in ACh release, as well as the behavioural effects induced by these agents. However, increased or decreased ACh levels in the hippocampus could causally induce alteration of some neuronal function by depressant and convulsant barbiturates, such as cognitive dysfunction and changes in consciousness, although this is pure speculation.

In summary, depressant R(−)-MPPB decreased, while convulsant S(+) MPPB increased, hippocampal ACh release in vivo in the rat, implying that changes in hippocampal ACh release may be related to the behavioural effects of barbiturates. The significance of these findings remains to be elucidated.

References
1 Ticku MK, Rastogi SK, Thyagarajan R. Separate site(s) of action of optical isomers of 1-methyl-5-phenyl-5-propylbarbituric acid with opposite pharmacological activities at the GABA receptor complex. Eur J Pharmacol 1985; 112: 1–9