Differential effects of ketamine and pentobarbitone on acetylcholine release from the rat hippocampus and striatum

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Summary
Using microdialysis, we examined the effects of ketamine and pentobarbitone on acetylcholine (ACh) release from the rat hippocampus and striatum. Ketamine 25 and 50 mg kg\(^{-1}\) increased ACh release from the hippocampus to 295% and 353% of basal release, respectively, but not from the striatum. SCH 23390 1 \(\mu\)mol litre\(^{-1}\), a D1 antagonist, significantly inhibited the facilitatory effect of ketamine 50 mg kg\(^{-1}\) on hippocampal ACh release (to 241% of basal level). In contrast, pentobarbitone 20 and 40 \(\mu\)g kg\(^{-1}\) decreased basal ACh release from both the hippocampus by 41% and 69%, respectively, and the striatum by 37% and 58%, respectively. The results suggest that ketamine and pentobarbitone exert opposite effects on ACh release from the rat hippocampus and that the stimulatory effect of ketamine may involve dopamine D1 receptors. (Br. J. Anaesth. 1996;77:381–384)

Key words

Glutamate receptors coupled to ion channels are classified into two major groups: those that are preferentially stimulated by the agonist N-methyl-D-aspartate (NMDA) and those that respond preferentially to other agonists (AMPA/kainate or non-NMDA receptors). It is generally accepted that ketamine, an i.v. general anaesthetic, is a non-competitive antagonist of NMDA receptors. Ketamine has been termed a “dissociative” anaesthetic because patients who receive ketamine alone appear to be in a cataleptic state. The effects of ketamine on the gross electrical activity of the brain show marked differences from that of “depressant” anaesthetics, such as pentobarbitone. Ketamine increases electrical activity in the brainstem reticular formation and limbic structures but the mechanisms are not yet fully understood.

Neurones releasing acetylcholine (ACh) are distributed widely in the brain and may be involved in memory, learning and motor functions. The hippocampus receives abundant extrinsic cholinergic innervation from the medial septal area which contains choline acetyltransferase-positive neurones. The septo–hippocampal cholinergic system is considered to be under the control of dopaminergic neurones. Indeed, several studies suggest that the hippocampus is regulated by dopamine activity, primarily at dopamine-1 (D1) receptors and that D1 agonists increase ACh release in the hippocampus. In contrast, the striatum is very important for the control of motor activity, and intrinsic neurones of the striatum are believed to be mostly GABAergic and cholinergic. The most important afferent projections appear to be the nigrostriatal dopaminergic pathway and the glutamatergic corticostriatal bundle. We hypothesize that the differences in anaesthetic mechanisms between dissociative and depressant anaesthetics may be because of different effects on ACh release in several brain regions.

The aim of this study was to investigate the effects of ketamine and pentobarbitone on hippocampal and striatal cholinergic neurones, and also to examine the relationship between ketamine and D1 receptor mechanisms in the rat hippocampus.

Materials and methods
We used the microdialysis technique in freely moving rats. The study was approved by the Animal Welfare Committee of Yokohama City University School of Medicine. We studied 61 male, adult Sprague–Dawley rats (weighing 250–350 g), anaesthetized with sodium pentobarbitone 40 mg kg\(^{-1}\) i.p. and placed in a stereotaxic apparatus (Model SR-6, Narishige Scient Instrument Lab., Japan). The guide cannula (CMA 10, Carnegie Medicine, Sweden) for penetration of a microdialysis probe was implanted stereotaxically into the right hippocampus (coordinates obtained from the bregma with the skull flat: A: −5.6 mm, L: 5.0 mm, V: 3.8 mm from the atlas of Paxinos and Watson) or the right striatum (coordinates from the bregma: A: 1.0 mm, L: 3.0 mm, V: 4 mm) and fixed in place with cranioplastic cement. After allowing at least 2 days for surgical recovery, microdialysis experiments were performed. The probe (CMA 11, Carnegie Medicine, Sweden) (3-mm long dialysis membrane, od 0.24 mm, molecular weight cut-off of 20 000 Da) was inserted into the guide cannula during light anaesthesia with diethyl ether. Microdialysis probes were perfused with Ringer’s solution containing eserine sulphate 10

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μmol litre⁻¹ (Wako Pure Chemical Industries Ltd, Japan), an inhibitor of choline esterase, at 2.0 μl min⁻¹, using a microdialysis pump (Model CMA 102, Carnegie Medicine, Sweden). Implanted probes were pre-perfused with Ringer’s solution for 120 min, and then every 20-min perfusate was collected in chilled polyethylene tubings containing 10 μl of ethylhomocholine 1 μmol litre⁻¹, an internal standard for HPLC measurement.

The test drugs used for these experiments were: ketamine 25–100 mg kg⁻¹ i.p. (Sankyo Ltd, Japan), pentobarbitone 20–40 mg kg⁻¹ i.p. (Abbott Laboratories, USA), MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate] 1 mg kg⁻¹ i.p. (RBI: Research Biochemical Incorporated, USA), a non-competitive antagonist of the NMDA receptor and non-anaesthetic agent, and SCH 23390 1 μmol litre⁻¹ (local infusion) (RBI: Research Biochemical Incorporated, USA), a D1 antagonist. A new rat was used for each drug dose tested. After four collections, ketamine, pentobarbitone and MK-801 were administered i.p. SCH 23390 was added to the perfusion solution from the start of experiments and continued throughout the experiments.

ACh release was measured by HPLC-ECD using a polymeric reversed-phase column (Eicom-pak AC-GEL, 6.0 x 150 mm, Eicom, Japan). An AC-Enzymap column (ACh esterase and choline oxidase-immobilized, Eicom, Japan) was used to convert ACh and choline to hydrogen peroxides, which were detected (Model ECD-100, Eicom, Japan) at 450 mV. The mobile phase was phosphate buffer 100 mmol litre⁻¹ (pH 8.5), containing tetra-sulphonic acid sodium salt 200 mg litre⁻¹, which were detected (Model ECD-100, Eicom, Japan) at 450 mV. The mobile phase was phosphate buffer 100 mmol litre⁻¹ (pH 8.5), containing tetra-sulphonic acid sodium salt 200 mg litre⁻¹, which were detected (Model ECD-100, Eicom, Japan) at 450 mV.

At the end of the experiments, rats were killed by overdose of diethyl ether, and the position of the probe in the brain was confirmed by visual examination. Percentage recovery of the dialysis probes was determined at the end of the experiment by dialysing a beaker containing known concentrations of ACh (here 1 μmol litre⁻¹) at 37 °C. Recovery in vitro = Cout/Cin, where Cout = concentration in the perfusate and Cin = concentration in the medium. Average ACh recovery through the probes at a flow rate of 2 μl min⁻¹ was 16.2 (SEM 1.5%) (n=11). Basal release was obtained from the mean of four initial collections before administration of test drugs and data are expressed as percentage of basal ACh release. The significance of differences between mean values was determined by ANOVA followed by Student’s t test. Data are mean, SEM.

**Results**

ACh release was stable for 120 min with baseline ACh concentrations in 20-min samples of 1.1 (SEM 0.2) pmol per sample from the hippocampus (n=39) and 6.7 (0.8) pmol per sample from the striatum (n=22).

Figure 1A and 1B show the effects of ketamine and pentobarbitone on ACh release from the hippocampus. Ketamine 25 and 50 mg kg⁻¹ i.p. increased ACh release from the hippocampus in a concentration-dependent manner. The mean maximum increases in ACh release by ketamine 25 and 50 mg kg⁻¹ i.p. were 295% and 353% of basal levels, respectively. Peak values of the mean increase were measured in the second sample after i.p. injection of ketamine 25 and 50 mg kg⁻¹ (fraction No. 6), and ACh release declined gradually. Ketamine 100 mg kg⁻¹ increased ACh release from the rat hippocampus more slowly, but ACh release remained elevated 120 min after i.p. administration (fig. 1A). There were no significant differences between the effects of ketamine 50 and 100 mg kg⁻¹ i.p. in the hippocampus.

MK-801 1 mg kg⁻¹ i.p. significantly increased ACh release from the rat hippocampus to 338 (22.5)% (n=4) of baseline. In contrast, pentobarbitone 20 and 40 mg kg⁻¹ i.p. decreased ACh release from the hippocampus in a concentration-dependent manner. Mean maximum decreases produced by pentobarbitone 20 and 40 mg kg⁻¹ i.p. were 41% and 69% of basal levels, respectively (fig. 1B).

In the hippocampus, SCH 23390 1 μmol litre⁻¹ (a D1 antagonist) significantly inhibited the facilitatory effect of ketamine 50 mg kg⁻¹ i.p. (peak value 241% of basal levels) (n=4), while SCH 23390 1 μmol litre⁻¹ alone failed to alter ACh release (fig. 2).

Figure 3A and 3B show the effects of ketamine and pentobarbitone on ACh release from the striatum. Ketamine 25, 50 and 100 mg kg⁻¹ i.p. did not affect ACh release, while pentobarbitone 20 and 40 mg kg⁻¹ i.p. decreased ACh release by 37% and 58% of basal levels, respectively.

**Discussion**

“In vivo” brain microdialysis techniques, coupled with a highly sensitive analytical method based on
HPLC-ECD, have recently made it possible to measure various neurotransmitters in freely moving rats. This permits a precise analysis of the effects of various anaesthetic agents on neurotransmission in the various brain regions. For example, previous studies indicated that diazepam reduced release of dopamine in both the nucleus accumbens and striatum of conscious rats, and morphine increased release of serotonin in the rat forebrain. Our study examined the differential effects of ketamine and pentobarbitone on ACh release from the hippocampus and striatum using in vivo microdialysis combined with HPLC-ECD.

Pentobarbitone decreased ACh release from the rat hippocampus and striatum, which may suggest a powerful "depressant" anaesthetic effect of pentobarbitone. Previous studies have shown that benzodiazepine agonists decreased ACh release in the hippocampus and striatum. In contrast, ketamine 25–100 mg kg\(^{-1}\) i.p. increased ACh release from the rat hippocampus. Our data demonstrated that the facilitatory effect of ketamine 100 mg kg\(^{-1}\) was slower and sustained, while that of ketamine 25 or 50 mg kg\(^{-1}\) was monophasic, although the underlying mechanisms remain to be determined.

Cocaine and amphetamine have also been shown to increase ACh release from the rat hippocampus. As cocaine and amphetamine are known to increase extraneuronal concentrations of endogenous dopamine in different dopaminergic areas, it appears that, in the hippocampus, ketamine may have a facilitatory role in controlling dopamine release. Furthermore, it might be suggested that these opposite effects of ketamine and pentobarbitone on ACh release from the rat hippocampus underlie the different properties of dissociative and depressant anaesthetics.

ACh release from the rat hippocampus may be linked to cognitive function. Cholinergic projections to the hippocampus originate from the perikarya in the medial septum and vertical limb of the diagonal band of Broca. Cholinergic activity in the hippocampus is increased during arousal, defined by behaviour or electroencephalography. Increased levels of hippocampal ACh accompanied by improved memory were demonstrated. It appears that elevated hippocampal cholinergic function may improve performance of certain cognitive tasks. A report that ketamine prevented the formation of flavour memories supports these findings. Ketamine may have a direct effect on learning or memory storage according to our data and this may be why patients anaesthetized with ketamine experienced bad dreams. Our study does not allow a clear conclusion as to the precise mechanism through which ketamine increases ACh release from the hippocampus. However, SCH 23390, a D1 antagonist, significantly attenuated the effect of ketamine on ACh release in our study, indicating that the facilitatory effect of ketamine might involve D1 receptor mechanisms, at least in part. Day and Fibiger, and Imperato, Obinu and Gessa demonstrated that cholinergic activity in the hippocampus is increased via D1 receptor mechanisms.

In the striatum, ketamine did not affect ACh release, while pentobarbitone decreased ACh release. We have reported that ACh release from the striatum was increased via D1 receptor mechanisms and decreased via D2/D3 receptor mechanisms. It might be suggested that ketamine has an important role in the regulation of ACh release from the rat hippocampal region, but not from the rat striatum, indicating regional selectivity between depressant anaesthetics such as pentobarbitone.

The highest density of NMDA receptors is found in the hippocampus. As MK-801 also increased ACh release in the hippocampus, NMDA block seems to underlie the effects of ketamine and is not simply a...
result of different anaesthetic properties. However, the in vivo effects of NMDA receptors on the activity of cholinergic neurones in the hippocampus are still poorly understood. Recent molecular evidence showed that block of NMDA receptors increased the effects of dopamine on D1 receptors 20. Considering these results, NMDA receptor antagonists, such as ketamine or MK-801, might be expected to increase ACh release from the rat hippocampus via D1 receptor mechanisms. Although our study of the effects of ketamine on ACh release needs further elaboration, it might help to explain the relationship between NMDA and D1 receptors in the rat hippocampus.

In summary, ketamine increased ACh release from the hippocampus, but not from the striatum, while pentobarbitone decreased ACh release from both the hippocampus and striatum. These results may underlie the differences between dissociative and depressant anaesthetics. Further, our data showed that the stimulatory effect of ketamine on ACh release from rat hippocampus may involve dopamine D1 receptors.

References