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Investigation into the effect of the general anaesthetics etomidate and ketamine on long-range coupling of population activity in the mouse neocortical slice

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ABSTRACT

General anaesthetics have been hypothesised to ablate consciousness by decoupling intracortical neural connectivity. We explored this by investigating the effect of etomidate and ketamine on coupling of neural population activity using the low magnesium neocortical slice model. Four extracellular electrodes (50 μm) were positioned in mouse neocortical slices (400 μm thick) with varying separation. The effect of etomidate (24 μM) and ketamine (16 μM) on the timing of population activity recorded between channels was analysed. No decoupling was observed at the closest electrode separation of 0.2 mm. At 4 mm separation, decoupling was observed in 50% and 42% of slices during etomidate and ketamine delivery, respectively ($P<0.0001$ and $P=0.002$, compared to 0.2 mm separation). A lower rate of decoupling was observed with 1 mm separation (21% and 8%, respectively, $P<0.03$ for etomidate compared to 0.2 mm separation). The data support the hypothesis that mechanistically diverse general anaesthetics disrupt neuronal connectivity across widely distributed intracortical networks.

1. Introduction

General anaesthetics have been used for more than 150 years to render patients unaware during surgical procedures. Despite their effective and widespread use, the mechanisms by which these drugs cause unconsciousness remain unknown. In part, this reflects our limited understanding of the neurophysiological processes in the brain that generate consciousness and the mechanisms by which the brain switches from conscious to unconscious states. Many theories have been presented to explain consciousness; however a unifying mechanism and a means of objectively measuring it remain elusive. Investigating the effects of general anaesthetics on the brain is a means not only to further our understanding of the mechanism of action of these drugs, but also increase our understanding of the neurophysiological correlates underpinning consciousness in the brain.

Much of the research into consciousness has focused on the cerebral cortex since this is where conscious perception is thought to be generated. Different functional areas within the brain are highly interconnected and create networks that allow for the complex integration and processing of stimuli (Martuzzi et al., 2010). Theoretical studies suggest that consciousness is generated through the ability of the cortex to integrate information across and within different areas (John and Prichep, 2005), and this integration may depend on coupling of neural activity over wide distances (Imas et al., 2006). One idea that has gained credence in recent years is that anaesthetics disrupt long-distance functional intracortical neuronal connectivity (Ferrarelli et al., 2010; Greicius et al., 2008; Koskinen et al., 2001; Martuzzi et al., 2010), which may be reflected in changes in neural coupling (Amzica and Steriade, 1995).

The aim of the present study was to investigate the effect of two anaesthetics, etomidate and ketamine, on neural connectivity by investigating coupling of neocortical population activity. We chose etomidate and ketamine because of their differing mechanisms of action, γ-aminobutyric acid (GABA) enhancement (Belelli et al., 1997) and *N*-methy-D-aspartate (NMDA) antagonism (Liu et al., 2001), respectively. We utilised the low-magnesium neocortical slice model because it is ideally suited for investigating intracortical mechanisms and it offers the advantage of high control of experimental conditions and ease of electrophysiological measurement. We hypothesised that both etomidate and ketamine

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would decouple population activity in mouse neocortical slices, pointing to a common mechanism of action of these two anaesthetics.

2. Methods

The Animal Ethics Committee at the University of Waikato approved all methods.

2.1. Preparation of slices

Neocortical slices were prepared from 7 to 10 week old female and male wildtype mice (C57/Bl6/129SV) housed under a 12 h light-dark cycle with free access to food and water. The mice were deeply anaesthetised with CO_2 and decapitated. For cerebral protection and conservation, the brain was rapidly removed and put in ice-cold artificial cerebrospinal fluid (aCSF). The aCSF was modified for cerebral protection according to Nowak and Bullier (1996) and contained 92.7 mM NaCl, 24 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 3 mM KCl, 19 mM $MgCl_2$, 0 mM $CaCl_2$ and 25 mM D-glucose, saturated with carbogen (95% O_2; 5% CO_2). The brain was sectioned into slices (400 μm coronal sections, between Bregma −2 to −5 mm) on a Vibratome (Campden Instruments, UK) in a bath of ice-cold protective aCSF as defined above. To recover, the slices were placed for a minimum of 1 h at room temperature (approximately 20 °C) in carbogenated no-magnesium aCSF, containing 124 mM NaCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 5 mM KCl, 0 mM $MgCl_2$, 2 mM $CaCl_2$ and 10 mM D-glucose. All solutions were made from double distilled water, stored at 1–4 °C and replaced after no more than one week.

2.2. Electrical recording

Recordings were made from single slices at room temperature in a bath perfused continuously with carbogenated no-magnesium aCSF delivered by either a peristaltic (P720, Instech Laboratories, USA) or syringe (50 ml) infusion (Terumo, Medtel, Australia) pump at a flow rate of 2.5 ml/min.

Four extracellular electrodes (50 μm teflon-coated tungsten wire) were positioned with 0.15–6.2 mm separation in the same hemisphere of the neocortex to record spontaneous local field potential activity. No particular cortical region was targeted. To ground the recording bath, a silver/silver-chloride disc electrode was used, which also served as a common reference. The recording apparatus was placed inside a Faraday cage to limit electrical noise. The neocortical signal was amplified (1000×, A–M Systems, USA) and analog bandpass filtered (high-pass 0.1 or 1 Hz, low-pass 100 Hz, notch 50 Hz) before analog-digital conversion at 1000 Hz (Power 1401, CED, UK) and recording on computer (Spike2, CED, UK) for later analysis.

2.3. Drug preparation and delivery

24 μM etomidate (Hypnomidate, Janssen–Cilag, Belgium) and 16 μM ketamine solutions were made by mixing the drug with carbogenated no-magnesium aCSF. The etomidate concentration was based on the known diffusion characteristics of etomidate into cortical slice tissue. At a recording depth of 100–200 μm, the slice concentration of etomidate is estimated to reach 17–50% of its maximum after 10 min perfusion of a constant aCSF concentration (Benkwitz et al., 2007). This would give a tissue concentration of approximately 1–3 μg/ml (4–12 μM). Deep anaesthesia with etomidate occurs at an estimated effect site concentration of 3.3 μg/ml in rodents (De Paepe et al., 1999), meaning the etomidate dose used in this study approximates a deep level of anaesthesia. The diffusion characteristics of ketamine into slice tissue have not been quantified. The lower concentration of ketamine used in this study was based on its higher clinical potency compared to etomidate. The anaesthetic solutions were delivered to the bath via the pump system described above.

2.4. Testing regimes

A stable pattern of population seizure-like event (SLE) activity, coupled (see definition in 2.5.2 below) across all four channels, was established in no-magnesium aCSF. Baseline activity was recorded for at least 10 min before continuing with the experimental protocol. Thereafter, etomidate (24 μM) or ketamine (16 μM) was delivered for 20 min, followed by a return to no-magnesium aCSF until SLE activity returned to a stable coupled pattern across all four channels. This protocol allowed each slice to act as its own control. Recordings were made from a total of 52 slices ($n=28$ for etomidate and $n=24$ for ketamine) from 26 animals. From these, six slices were excluded from the analysis on the basis of technical difficulties ($n=4$) or non recovery of SLE activity after etomidate washout ($n=2$).

Additional control data were collected from six slices (four animals) in which no-magnesium aCSF was run for approximately 90 min without the addition of anaesthetic.

To investigate the effect of physical cortical disconnection, recordings were made from slices ($n=5$) in which two electrodes were positioned within the same hemisphere (3–4 mm separation) and SLE activity recorded before and after a physical cut was made through the depth of the cortex midway between the electrodes. The data was analysed over 5 min windows before and after cutting and quantified as the percentage of coupled activity according to the criteria set out in the following section.

2.5. Data analysis and statistics

Data were analysed using MATLAB (Version 7.3.0.267 (R2006b), The Mathworks Inc., Natick MA USA) and GraphPad InStat 3 software (Version 3.06, 32 bit for Windows, GraphPad Software, San Diego, CA USA). All of the recordings were visually inspected and identified artefacts were manually removed. Out of 28 etomidate slices, six had one channel each excluded due to activity dying out ($n=2$) or too much noise for accurate analysis ($n=4$). For the 24 ketamine recordings, one channel consistently failed to record SLE activity (due to a damaged wiring connection) and was excluded from all analyses. A further three recordings had one additional channel excluded due to electrical noise. For all of the parameters investigated, data were averaged over 5–10 min windows before, during and after anaesthetic delivery. In the control slices only two time windows, 10 and 50 min into the recording (representing before and after anaesthetic delivery, respectively), were compared. Data normality was assessed using the Kolmogorov–Smirnov test and, unless otherwise stated, expressed as median (interquartile range). For the analyses described below, parametric or nonparametric repeated measures or paired tests were used according to whether the data was shown to be normally distributed. Statistical significance was set at $P<0.05$.

2.5.1. SLE frequency and amplitude

The frequency of SLE activity was calculated throughout each recording as a moving average, with a time window of two minutes and an overlap of 50 s. SLE amplitude was calculated as the peak-to-peak amplitude. To ensure that the statistical analysis was robust we used a randomised channel set (one channel randomly selected from each slice) because activity patterns tended to be correlated within a slice. The effect of etomidate