Anaesthetic Research Society

Vacation Studentship Report 2010

Title

Identification of the Molecular Interactions of Xenon with the NMDA Receptor Glycine Site

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BACKGROUND

Background. The general anaesthetic inert gas xenon inhibits NMDA receptors(*1, 2*) and has been shown to be neuroprotective in *in vitro* and *in vivo* models(*3-5*). Xenon is currently undergoing early clinical trials for neuroprotection in neonatal ischemia & cardiopulmonary bypass. However, the molecular mechanism of xenon's neuroprotection remains to be elucidated. We have recently shown that xenon inhibits the NMDA-receptor by competing with the co-agonist glycine(*6*) (see Figure 1). This is the first time that a specific anaesthetic binding-site has been identified on the NMDA-receptor. This mode of NMDA-receptor inhibition may explain why xenon has beneficial clinical properties (e.g neuroprotection, profound analgesia) while certain other anaesthetics do not. Certain drugs that inhibit NMDA receptors have neuroprotective properties. However, many of these NMDA receptor antagonists are also neurotoxic (*e.g.* ketamine, MK801, nitrous oxide). Our recent observation that xenon competes at the glycine site of the NMDA receptor may have important implications for general anaesthesia and neuroprotection. Neuroprotective NMDA-receptor glycine site antagonists (*e.g.* Gavestinel) are well tolerated in patients and devoid of psychotomimetic side effects common with many NMDA receptor antagonists.

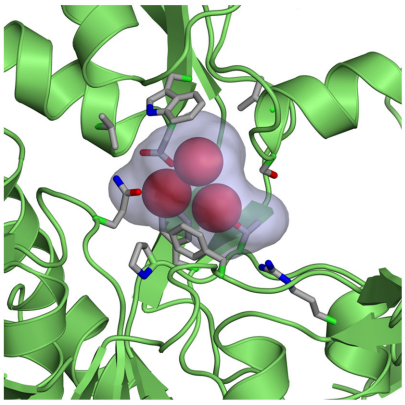

Figure 1. We have identified 12 amino-acids likely to interact with xenon at its binding site in the NMDA-receptor glycine site.

REPORT ON RESEARCH UNDERTAKEN

Aims & Hypothesis. Our molecular modelling identified 12 amino-acids(*6*) in the glycine binding site of the NMDA receptor likely to interact with xenon. The project aims to determine which of these amino acids are involved in inhibition by xenon and isoflurane. We have mutated these amino acids using site-directed mutagenesis. As a first stage we have already determined glycine-affinity of 4 of the mutant receptors. The summer project student will determine the sensitivity of these mutant receptors to xenon. Using patch-clamp electrophysiology we will perform inhibition measurements at different glycine concentrations. Our hypothesis is that the mutations will reduce or eliminate the xenon's competitive inhibition.

Methods

Site-directed mutagensis was used to mutate specific amino-acids in the glycine site of the NMDA receptor NR1 subunit. The calcium-phosphate transfection method was used to express the mutant NR1 subunits together with wild-type NR2A subunits of the NMDA receptor in human embryonic kidney (HEK 293) cells. The HEK cells were co-transfected with green fluorescent protein to allow identification of successfully transfected cells for electrophysiology. Patch-clamp electrophysiology using the whole cell recording configuration was used to assay NMDA receptor function. A double-barreled rapid perfusion system was used to expose the cells to the experimental solutions and to perform switches in and out of NMDA-containing solutions. NMDA-evoked currents in the absence and presence of anaesthetic were recorded at a range of different glycine concentrations.

Results & Discussion

Inhibition of mutant and wild-type (WT) receptors by xenon were compared at a range of glycine concentrations. We showed that in WT receptors xenon inhibition increases as the glycine concentration is decreased, consistent with xenon competing with glycine at the glycine site. However, in the NR1 Phe758Leu and Phe758Ala mutants to the glycine dependence of the inhibition is abolished. These findings provide additional evidence that xenon binds to the glycine site of the NR1 subunit of the NMDA receptor. The attenuation of xenon inhibition of the Phe758Leu and Phe758Ala mutants suggests that an interaction between xenon and the aromatic ring of the phenylalanine residue is important in the xenon binding to the WT NMDA receptors

Dissemination

The work was presented at the winter meeting of the ARS at the Royal College of Anaesthetists in London in November 2010. It will be published as an abstract in the British Journal of Anaesthesia in 2011

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