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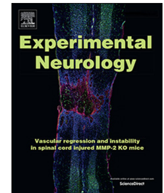

Research paper

Ketamine reduces deleterious consequences of spreading depolarizations

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ABSTRACT

Recent work has implicated spreading depolarization (SD) as a key contributor the progression of acute brain injuries, however development of interventions selectively targeting SD has lagged behind. Initial clinical intervention efforts have focused on observations that relatively high doses of the sedative agent ketamine can completely suppress SD. However, blocking propagation of SD could theoretically prevent beneficial effects of SD in surrounding brain regions. Selective targeting of deleterious consequences of SD (rather than abolition) could be a useful adjunct approach, and be achieved with lower ketamine concentrations. We utilized a brain slice model to test whether deleterious consequences of SD could be prevented by ketamine, using concentrations that did not prevent the initiation and propagation of SD. Studies were conducted using murine brain slices, with focal KCl as an SD stimulus. Consequences of SD were assessed with electrophysiological and imaging measures of ionic and synaptic recovery. Under control conditions, ketamine (up to 30 μM) did not prevent SD, but significantly reduced neuronal Ca^{2+} loading and the duration of associated extracellular potential shifts. Recovery of postsynaptic potentials after SD was also significantly accelerated. When SD was evoked on a background of mild metabolic compromise, neuronal recovery was substantially impaired. Under compromised conditions, the same concentrations of ketamine reduced ionic and metabolic loading during SD, sufficient to preserve functional recovery after repetitive SDs. These results suggest that lower concentrations of ketamine could be utilized to prevent damaging consequences of SD, while not blocking them outright and thereby preserving potentially protective effects of SD.

1. Introduction

Spreading depolarization (SD) is a slowly propagating wave (2–4 mm min^{-1}) of near-complete neuronal and glial depolarization that has gained renewed interest as an important contributor to the progression of acute brain injuries (Lauritzen et al., 2011; Dreier et al., 2017; Hartings et al., 2017). SD can be initiated by stimuli that cause synchronous depolarization of a critical volume of brain tissue (Tang et al., 2014), and SD propagation across the brain is propelled by feed-forward release of glutamate and/or K^+ (Somjen, 2001). In injured brain, the initiating depolarization is caused by ischemia, trauma, or other energetic supply-demand mismatches (von Bornstadt et al., 2015). The extent of ionic loading accompanying SD is extreme, with intracellular Ca^{2+} loads continuously exceeding 10s of micromolar for more than a minute (Somjen, 2001; Dietz et al., 2008). As such, the metabolic costs to recover from SD are much more demanding than other brain phenomena, such as seizures (Dreier et al., 2013), and thus are particularly challenging for the injured brain (Hartings et al., 2017).

Whether or not injury occurs after SD depends greatly on the capacity of tissues to re-establish ionic gradients in the aftermath of SD. This capacity is influenced by the degree of ionic loading during SD, the baseline metabolic status, and the ability of a region to profoundly increase blood flow to match energy demands after SD (Dreier, 2011). This is exemplified during SD in the healthy brain (e.g. migraine aura), where metabolic and vascular perfusion reserves are adequate, and thus SD does not result in any permanent damage (Nedergaard and Hansen, 1988). In contrast, SDs that spontaneously occur following stroke (Dohmen et al., 2008), trauma (Hartings et al., 2011), or subarachnoid hemorrhage (Dreier et al., 2009) can underlie stepwise progression of injury (Busch et al., 1996; Hartings et al., 2003, 2017).

The development of clinical interventions for SD has lagged behind efforts to demonstrate their incidence in different pathologic conditions. Initial efforts have concentrated on the application of agents such as NMDA receptor (NMDAR) antagonists that block the initiation and propagation of SD. The dissociative anesthetic ketamine is an NMDAR antagonist that prevents SD in animal models (Hernandez-Caceres et al., 1987; Marrannes et al., 1988) and shows effectiveness in case reports (Sakowitz et al., 2009; Schiefecker et al., 2015). A retrospective review of medications used in the intensive care unit (ICU) also shows that ketamine infusion can reduce the frequency of SDs in brain injured

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patients (Hertle et al., 2012). Prospective studies of ketamine would be useful to determine whether a reduction in SD frequency is associated with improved outcomes in the clinic. However, such studies are complicated by two potential problems. First, the high ketamine concentrations used to suppress SD also result in substantial sedation with attendant increases in risk of ICU complications (Abou-Chebl et al., 2010; Nichols et al., 2010). Secondly, SDs propagate widely in injured brain, including through tissue that may be distant from an injury core where intact metabolic capacity is retained. It is possible that SDs invading these distant regions cause protective preconditioning (Yanamoto et al., 2004; Viggiano et al., 2016), adaptive synaptic plasticity (Faraguna et al., 2010), and/or neurogenesis (Urbach et al., 2017) that may be beneficial to functional recovery (Nakamura et al., 2010; Dreier, 2011). These theoretical issues require further study, but suggest that different approaches to selectively target the deleterious consequences of SD could be a useful adjunct to the current focus on global block of SD events.

We tested here whether deleterious effects of SD could be limited by lower concentrations of ketamine that do not prevent SD outright. Our findings with measurements of Ca^{2+} loading and a model of metabolic vulnerability indicate that ketamine can be protective without blocking SD, and support a possible significant modification of therapeutic strategies for SD, based on blocking consequences of SD rather than incidence.

2. Materials and methods

2.1. Animals and preparations

All animal procedures were performed in accordance with protocols approved by the UNM Health Sciences Center Institutional Animal Care and Use Committee. Adult (4–8 weeks) male and female mice (C57Bl/6 and/or GCaMP5G) were used for all experiments. For Ca^{2+} imaging experiments, homozygous mice expressing the floxxed calcium indicator GCaMP5G under the CAG promoter (Gee et al., 2014) were purchased from The Jackson Laboratory (Stock No: 024477, B6;129S6-*Polr2a*$^{tm1(CAG-GCaMP5g,-tdTomato)Tvrd}$/J), and bred with homozygous mice expressing Cre Recombinase under the CamK2a promoter (B6.Cg-Tg (Camk2a-cre)T29-1Stl/J, Jax Stock No: 005359). Offspring were utilized in experiments and had robust GCaMP5G expression in hippocampal pyramidal neurons (Wang et al., 2013).

Acute brain slices were prepared as previously described (Shuttleworth et al., 2003). Briefly, animals were deeply anaesthetized with 0.15 mL (s.c.) injection of ketamine-xylazine (85 and 15 mg/ml, respectively), decapitated, and brains were quickly removed into 150 mL oxygenated ice-cold cutting solution (in mM): sucrose, 220; $NaHCO_3$, 26; KCl, 3; NaH_2PO_4, 1.5; $MgSO_4$, 6; glucose, 10; $CaCl_2$ 0.2; equilibrated with 95% O_2/5% CO_2 supplemented with 0.2 ml ketamine (100 mg/ml, Putney Inc., Portland, ME), to limit excitotoxicity during the slice preparation as described in (Aitken et al., 1995). Coronal cortico-hippocampal slices (350 μm) were prepared with a Pelco 102 Vibratome (Ted Pella, Inc., Redding, CA), hemisected, and then allowed to recover in artificial cerebrospinal fluid (aCSF; containing (in mM): NaCl, 126; $NaHCO_3$, 26; glucose, 10; KCl, 3; $CaCl_2$, 2, NaH_2PO_4, 1.5; $MgSO_4$, 1; equilibrated with 95% O_2/5% CO_2), at 35 °C for 60 min. After 1 h, the holding aCSF was replaced with chilled (20 °C) aCSF and slices were allowed to equilibrate to room temperature until the start of recording sessions. These incubations and exchanges served to ensure effective wash out of residual ketamine from slices, as previously established with responsiveness to glutamate and NMDA (Shuttleworth et al., 2003; Hoskison and Shuttleworth, 2006; Vander Jagt et al., 2008).

2.2. Generation of SD

Individual brain slices were transferred to a submersion recording chamber with nylon slice supports (RC-27 L, Warner Instruments, Hamden, CT), and continuously super fused with oxygenated (95% O_2/95% CO_2) aCSF at 2.2 ml min^{-1}. Bath temperature was maintained at 32 °C by an inline heater assembly (TC-344B, Warner Instruments). After placement of electrodes into the slice (See Electrophysiology methods) slices were allowed 20 min for equilibration. As described below (Results), modified aCSF with elevated K^+ (8 mM) was used for most experiments, in order to increase ability of single slices to support repetitive SDs and enable rigorous testing of drug effects (Funke et al., 2009; Zhang et al., 2015). SDs were evoked by pressure microinjection (40 ms, 30 psi, Picospritzer; Parker Hannifin, OH, USA) of KCl (1 M) via a glass micropipette ~3 MΩ placed in hippocampal CA1 stratum radiatum. Repetitive SDs were initiated in each slice at 15 min intervals to allow for full recovery between events. In experiments assessing the effect of ketamine antagonism during repetitive SDs (Figs. 2–4 & Supplementary Figures), antagonist wash-in commenced following the second of two control SDs, and the second control SD was used for analyses (Footitt and Newberry, 1998). SD initiation and propagation, as well as slice viability (see Metabolic challenge below), were examined by monitoring intrinsic optical signals (IOS) of submerged brain slices trans-illuminated with visible light (≥600 nm) and collected using a 4× objective (Olympus, 0.10 NA). IOS data were captured at 0.5 Hz using a cooled CCD camera (Imago, Till Photonics) and analyzed with TillVision software (TillPhotonics, version 4.01). Data analysis involved normalizing transmitted light to baseline and expressing IOS as percent change in transmission ($\Delta T/T_0 \times 100$) (Anderson and Andrew, 2002).

2.3. Electrophysiology

Extracellular recordings were acquired (1–10 kHz) with an Axon MultiClamp 700A amplifier, digitized (Digidata 1332), and recorded using pCLAMP10.2 software (Molecular Devices, Sunnyvale, CA, USA). Glass recording microelectrodes were filled with aCSF (tip resistance ~3MΩ) and positioned at a depth of 50–100 μm in the CA1 stratum radiatum ≥200 μm from the KCl-filled glass ejection micropipettes. The durations of SDs were calculated from the extracellular potential shift ("DC shift" (Somjen, 2001)), measured at 20% of the peak maximum to 80% recovery. In experiments assessing synaptic recovery after SD, a concentric bipolar electrode (FHC, Bowdoin, ME, USA) was placed on the slice surface ofCA1 stratum radiatum, between the KCl ejection micropipette and recording electrode, for stimulation of Schaffer collateral inputs. Excitatory postsynaptic potentials (EPSPs) were recorded using test pulses (50 μs, 0.1 Hz) delivered at intensities (80–400 μA) that gave 40–60% of the maximum EPSP amplitude. DC shifts and EPSPs were analyzed using Clampfit 10.2 software (Molecular Devices, Sunnyvale, CA, USA). Postsynaptic potentials were resolved from gap-free recordings with a high-pass filter (1 Hz cut-off). The duration of EPSP suppression after a single SD was measured from the time of the maximum negative potential of the DC shift to the time at which postsynaptic potentials first reached ≥50% of baseline values.

2.4. Fluorescence imaging

Neuronal Ca^{2+} dynamics during SD reported by GCaMP5G were imaged with a 20× water-immersion objective (Olympus, 0.5 NA) and analyzed in TillPhotonics, version 4.01 software (Till Photonics GmbH, NY). GCaMP5G was excited at 480 nm using a monochromator (Polychrome V, 2 Hz); emission signals were passed through a dichroic mirror (515 DCLP) and captured using a cooled CCD camera (Imago, Till Photonics). Total Ca^{2+} accumulation in specific regions of interest during SD were calculated (GraphPad Prism 7.03) as the integral of the signals for 120 s or 200 s following the peak of the SD transient. The duration of Ca^{2+} during SD was measured from the initial positive peak amplitude to the time point where fluorescence returned to ≤5% of baseline levels.

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