A novel slow γ-aminobutyric acid type-A receptor anesthetic KSEB 01-1 preserves mouse astrocyte mitochondrial dynamics after glucose deprivation injury.

SAPTAPARNI HAZRA[1], XIAOYUN SUN[1], ANDRES MALDONADO[1], BHAVANA KUNISETTY[1], NATHANEAL SMITH[1], XIAOYAN ZHANG[1], FRANCES DAVIES[2], EDWARD BERTACCINI[2] AND CREED M. STARY[1]

1.Dept. Anesthesiology, Perioperative and Pain Medicine, Stanford University, Stanford, CA, USA 2. Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA

INTRODUCTION

- **KSEB-01-1** is a structurally distinct N-arylpyrrole anesthetic designed to preferentially target slow/tonic **GABA_A receptors**.
- We hypothesized that selective activation of these receptors by **KSEB-01-1** would promote **mitochondrial fusion during and after metabolic injury**, compared with propofol.
- Mitochondrial function depends on the balance between **fusion and fission**: fusion (MFN2, OPA1) preserves mitochondrial structure and respiration, while fission supports quality control.
- In this study, we evaluated the **dose-dependent effects of KSEB-01-1 and propofol on the mitochondrial fusion proteins MFN2 and OPA1** in primary astrocytes under baseline conditions and during recovery from metabolic injury.

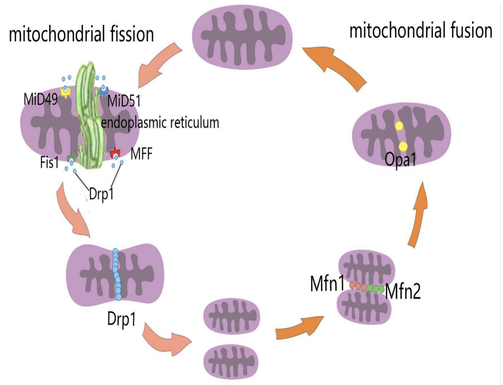


Fig1: Mitochondrial dynamics in astrocyte and neuronal cultures. **Fusion**: promotes the exchange of contents between mitochondria, preventing the accumulation of defective components. Critical in mitochondrial biogenesis and trafficking. **Fission**: helps to segregate and remove damaged or dysfunctional mitochondria, contributing to mitochondrial quality control.

METHODS,

1. After Stanford IRB approval, we evaluated the effects of the novel anesthetic **KSEB 01-1** and **propofol** on mitochondrial dynamics in primary astrocyte cultures.

2. Primary cortical astrocytes from neonatal mice were treated with KSEB 01-1 or propofol for 24 h at multiple doses (5, 10, and 30 μM) to assess baseline effects on mitochondrial fusion proteins **MFN2** and **OPA1**.

Workflow:

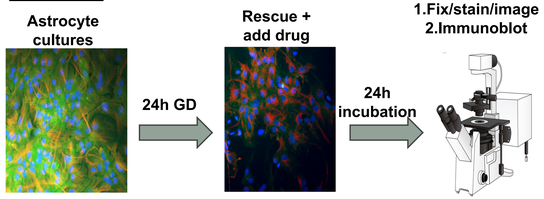
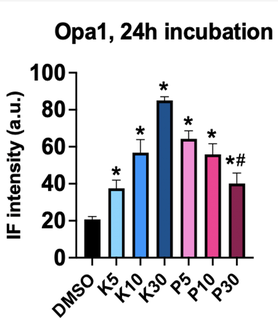


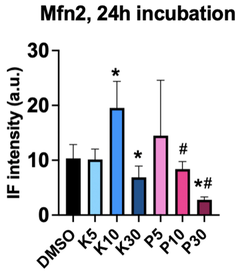
Fig 2: Experimental Design for Assessing Anesthetic Effects on Astrocyte Mitochondrial Dynamics

3. To model metabolic injury, astrocytes underwent **24 h glucose deprivation**, followed by resuscitation with KSEB 01-1 or propofol during the recovery phase.

4. After 24 h of recovery, cells were analyzed by **MFN2 immunofluorescence imaging** or **immunoblotting** for protein quantification.

RESULTS



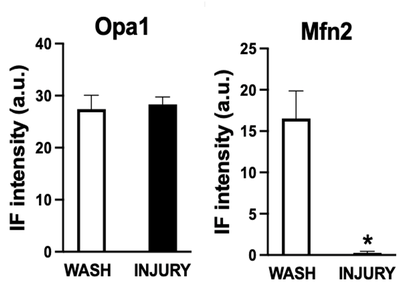


* = p<0.05 versus DMSO
\# = p<0.05 versus KSEB

Fig 3: Effect of 24h anesthetic incubation on mitochondrial fusion proteins

Figure 3a (top) OPA1: Increased significantly with both **KSEB 01-1** and **propofol** at all doses ($p < 0.05$), with the highest increase at **KSEB 30 μM**.

Figure 3b (bottom) MFN2: Slight rise at **KSEB 10 μM** but decreased at **KSEB 30 μM** and with all **propofol** doses.



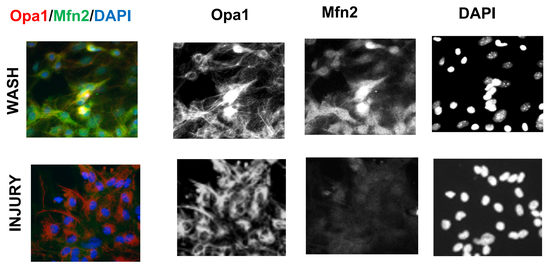
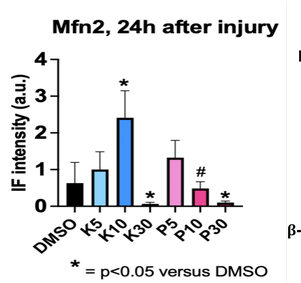


Fig 4. Effect of glucose deprivation (GD) injury on mitochondrial fusion proteins in primary astrocytes. Representative immunofluorescence images (right) of astrocyte cultures under **wash control** conditions and **24 h after GD injury**. The merged image shows **MFN2 (green), OPA1 (red), and DAPI nuclear stain (blue)**, followed by the individual fluorescence channels for **OPA1, MFN2, and DAPI.(right)** Quantitative analysis and immunoblot confirmation demonstrate that **GD injury alone results in a significant decrease in MFN2 with no change in OPA1**, indicating a selective reduction of MFN2 following metabolic injury.



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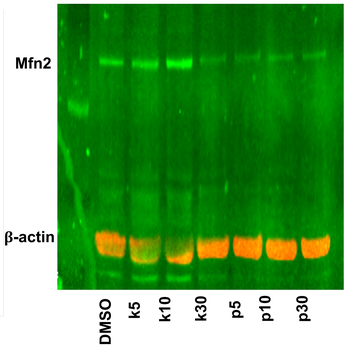

Fig 5: KSEB 01-1 preserves MFN2 expression in astrocytes during recovery from metabolic injury. Representative immunoblot (right) and fluorescence-based quantification (left) of **MFN2 expression** in primary astrocyte cultures 24 h after glucose deprivation (GD) injury followed by treatment with KSEB 01-1 (5, 10, 30 μM), or propofol (5, 10, 30 μM) during the recovery phase. MFN2 protein levels were normalized to β-actin. Quantification of MFN2 immunofluorescence intensity shows that **KSEB 01-1 (10 μM)** significantly **preserved MFN2 expression** compared with DMSO controls, whereas **propofol did not preserve MFN2 at any concentration**.

CONCLUSIONS

1. In primary astrocyte cultures recovering from glucose deprivation, both KSEB 01-1 and propofol increased OPA1; however, only low-dose KSEB 01-1 (10 μM) elevated MFN2 after 24 h and when given during recovery.
2. This divergence aligns with KSEB 01-1's preferential engagement of slow/tonic GABA_A receptors, shifting mitochondrial dynamics toward fusion.
3. Overall, **KSEB 01-1 shows a fusion-preserving advantage after metabolic injury**.

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