

Sevoflurane causes widespread increase in PKMζ in the CA1 pyramidal cells of the hippocampus in adult male mice: Implications for Post-Operative Cognitive Dysfunction.

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INTRODUCTION & BACKGROUND:

Post-Operative cognitive dysfunction (POCD) is a frequent complication after anesthesia that significantly affects patients’ postoperative quality of life and well-being. Although it is well recognized that elderly individuals are more vulnerable to developing POCD, the underlying mechanisms responsible for POCD are not fully understood. Recent studies have implicated neuroinflammation in the CA1 region of the hippocampus in the occurrence of POCD (1) . Additionally, persistent increases of the atypical protein kinase C isoform, PKMζ, have been shown to maintain long-term memory traces in the CA1 region of the hippocampus (2). Brief exposure to sevoflurane causes preconditioning in rat hippocampal slices in part through new synthesis of PKMζ, which is critical for sevoflurane-induced neuroprotection (3). Neurons thus appear capable of differentiating between two pools of PKMζ molecules: 1) those maintaining memory traces, and 2) those mediating neuroprotection. We hypothesize that if this ability is compromised due to aging or pathology, sevoflurane might cause mixing of the two PKMζ pools, introducing “noise” into a patient’s memory and contributing to POCD. Whereas persistent increases in PKMζ maintain memory in hippocampus (2), whether sevoflurane also increases PKMζ in the hippocampus *in vivo* under conditions similar to patient exposure during anesthesia is unknown. We therefore set out to test whether *in vivo* exposure to sevoflurane affects PKMζ expression in the hippocampus as well. Using immunohistochemistry (IHC), we examined whether increased expression of PKMζ follows a 2-hour exposure of 4% sevoflurane in mice *in vivo*.

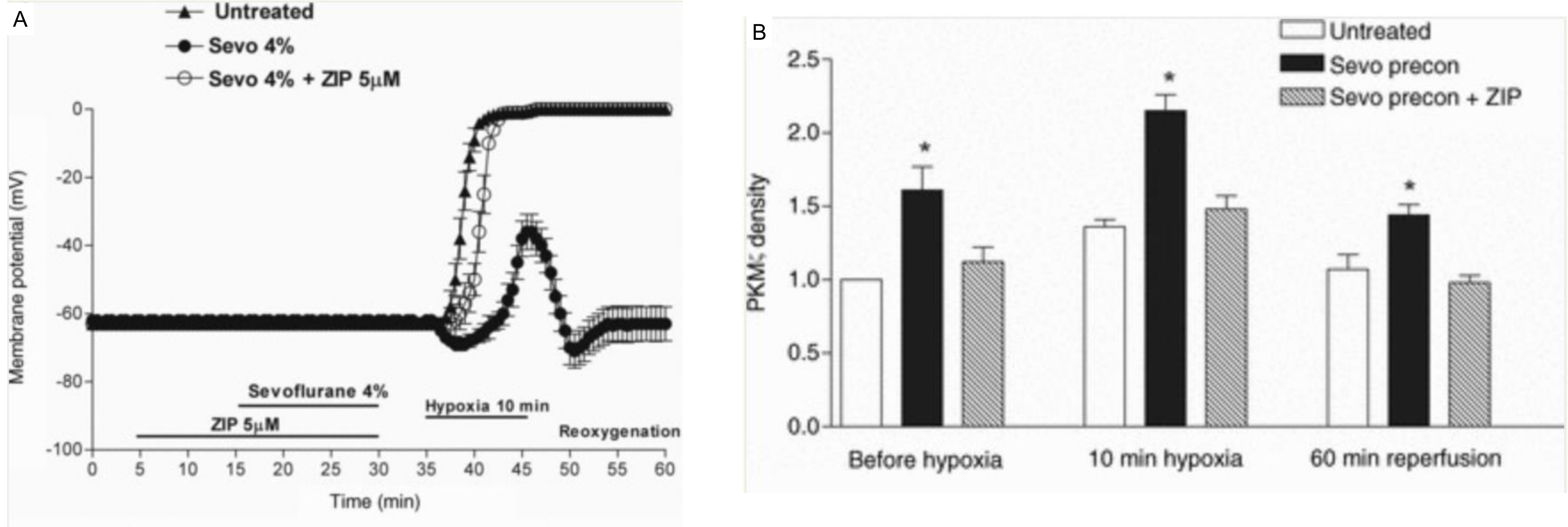


Figure 1. (A) Effect of zeta inhibitory peptide (ZIP), an atypical PKC inhibitor, and sevoflurane-induced preconditioning on electrophysiological responses during and after 10 min of hypoxia. Resting potentials are recorded from CA1 pyramidal cells before, during, and after 10 min hypoxia. In untreated slices (no sevoflurane) there is no recovery of the resting potential after hypoxia. When 4% sevoflurane is given via a calibrated vaporizer in the gas stream for 15 min and then washed out for 5 min before hypoxia, the slices show an enhanced hyperpolarization, a delayed and attenuated depolarization, and recovery of the resting potential after hypoxia. The figure shows the potential for 15 min after hypoxia; and the statistics are from 60 min after the hypoxia. When 5 μm ZIP is applied 10 min before and during the sevoflurane treatment, the effects of sevoflurane on the electrophysiological changes are blocked. ANOVA followed by a *post hoc* Student–Neuman–Keul test was used to analyze the significant difference among different experimental groups. All the data are expressed as mean ± SEM. The difference between different groups was considered significant if *P* < 0.05. **(B) Effect of ZIP and sevoflurane-induced preconditioning on PKMζ protein levels before, during and after 10 min of hypoxia.** Slices are either untreated, subjected to sevoflurane-induced preconditioning, or ZIP 10 min before sevoflurane-induced preconditioning. For each Western blot an untreated non-hypoxic control is run on the same gel and used as a normalization standard; its density is set to 1 and all other bands are a ratio of this density. Six CA1 regions from tissue treated in the same chamber are from a single animal and are microdissected and pooled to run in a single lane. Each bar represents the mean and SEM from 10 animals. Sevoflurane-induced preconditioning significantly increased the amount of PKMζ protein before, during, and after hypoxia. ZIP reduced the amount of PKMζ protein at these time points. * *P* < 0.05 compared with untreated group. **(Figures from Wang et al 2012)**

METHODS:

2–4-month-old male mice were exposed to 4% sevoflurane in O₂ for 2 hours, or O₂ alone, and brains were fixed by cardiac perfusion. Hippocampal slices were placed in ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) immediately and post-fixed for 48 hr. Slices were then washed with PBS (pH 7.4) and cut into 40 μm sections using a Leica VT 1200S vibratome. As previously described (4), free-floating sections were permeabilized with PBS containing Triton X-100 [PBS-TX100] for 1 hr at room temperature and blocked with 10% normal donkey serum in PBS-TX100 (Blocking Buffer, BB) for 2.5 hr at room temperature. The sections were then incubated overnight at 4°C with primary antibody rabbit anti-PKMζ C-2 antisera (1:10,000) in BB. After washing six times for 10 min each in PBS-TX100, the sections were then incubated with biotinylated donkey anti-rabbit secondary antibody (1:250 in BB; Jackson ImmunoResearch) for 2 hr at room temperature. After washing six times for 10 min each in PBS-TX100, the sections were incubated with streptavidin conjugated-Alexa 647 (1:250 in PBS-TX100; Jackson ImmunoResearch) for 2 hr at room temperature. After extensive washing with PBS-TX100, the sections were mounted with 4′,6-diamidino-2-phenylindole (DAPI) Fluoromount-G (Southern Biotech) or Vectashield (Vector Laboratories), and imaged using an Olympus FluoView FV1000 or Zeiss LSM 800 confocal microscope. All parameters (pinhole, contrast, and brightness) were held constant for all sections from the same experiment. To compare the intensity profile of PKMζ-immunostaining between images, we converted the images into grayscale and used the MATLAB functions “*imread*” and “*imagesc*” to scale the intensity distribution into the full range of a colormap.

RESULTS:

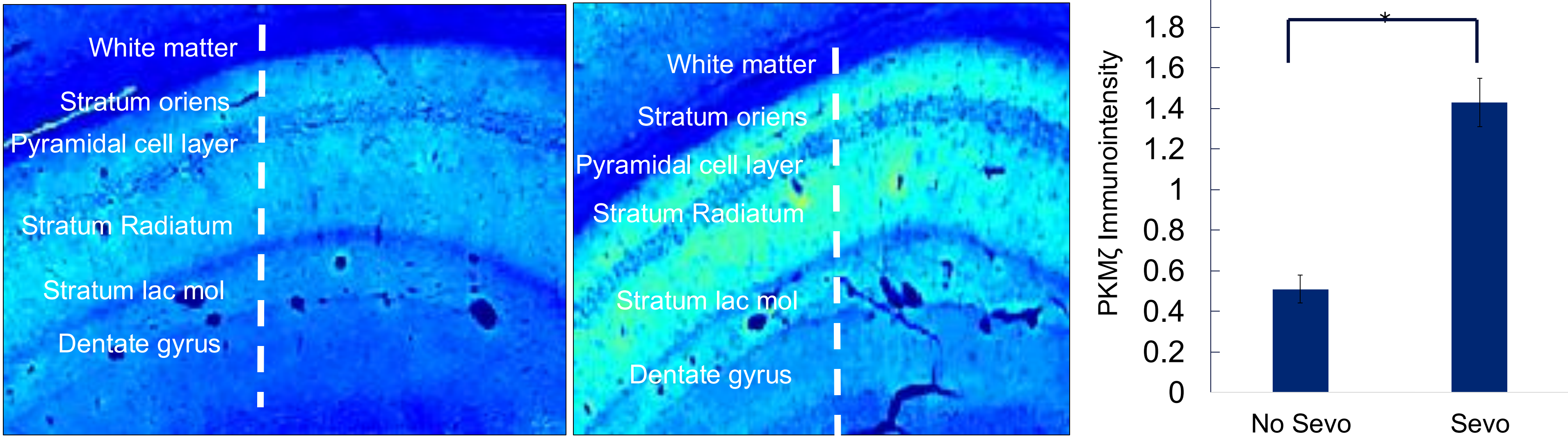


Figure 2: Sevoflurane preconditioning causes widespread increase of PKMζ in CA1 pyramidal cells of adult male mice. IHC results after 4% sevoflurane exposure show that PKMζ expression increases in sevoflurane treated mice. The ANOVA reveals the significant difference of the immunointensity of PKMζ in CA1 *str. radiatum* among the two groups (Sevo treated and non-Sevo treated). PKMζ expression was quantified by comparing the ratios of immunointensity in the *str. radiatum* to that of the white matter for each sample.

CONCLUSION:

POCD is a common post-operative complication in surgical patients. A recent meta-analysis showed that, among the numerous volatile anesthetics studied, sevoflurane had the worst effect on reducing the incidence of POCD (5). Our data show that sevoflurane increases PKMζ expression in the hippocampus, which may help explain the mechanism by which sevoflurane causes POCD in some patients. These findings can guide future efforts in understanding and reducing the incidence of POCD.

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