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Agmatine inhibits NMDA Receptor-mediated Calcium Transients in Spinal Cord Dorsal Horn

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Aims, and Hypothesis: The activation of N-methyl-D-aspartate receptors (NDMARs) in the spinal cord dorsal horn, and the subsequent Ca^{2+} permeation into neurons, are established contributions to chronic pain. Specifically, antagonism of the GluN2B subunit-containing NMDARs (GluN2B-NMDARs) is involved in the development of chronic pain. Our previous studies have shown that the intrathecal administration of Agmatine, an endogenous decarboxylated form of L-arginine that selectively antagonizes GluN2B-NMDARs, inhibits and reverses the pain behaviors in animal models of chronic pain. However, the mechanism of Agmatine's inhibition on Ca^{2+} transients in the spinal cord dorsal horn is still unknown.

This study aims to define the modulatory effect of agmatine on NMDARs-mediated Ca^{2+} transients in ex vivo mouse spinal cord slices. **The hypothesis** is that 1) agmatine can concentration-dependently inhibits NDMARs-mediated Ca^{2+} transients, similar to other NMDAR antagonists. 2) agmatine's inhibitory effect can be reversed by GluN2B knock-down.

Methods: Female and male ICR mice (4-6 weeks) were perfused before spinal cord extraction, and ex vivo spinal slices were incubated with the calcium indicator dye Fluo-4. For GluN2B knock-down mice (GluN2B-KD), intraspinal injections of Cre-containing AAV9-hSyn-GCaMP6s-cre and control AAV9-hSyn-GCaMP6s-Δcre viruses were performed on C57 GluN2Bfl/fl mice. Both viruses encoded a Ca^{2+} indicator, GCaMP6s, to visualize Ca^{2+} transients. 4 weeks after injection, laminectomy was conducted and spinal neurons expressing Cre recombinase were distinguished by GCaMP6s expression. Intracellular Ca^{2+} was visualized by single-plane two-photon microscopy. Time-lapse of images were acquired and the peak amplitude of fluorescence intensity was analyzed by Student's t-test.

Results: 2 minutes incubation of APV (2, 10, 50 μM), ifenprodil (30, 100, 300 μM), and Agmatine (1, 3.3, 10 mM) concentration-dependently attenuated the NMDARs-mediated Ca^{2+} transients. For ifenprodil, we also found that longer incubation (15 min) resulted in significantly higher inhibition of NMDAR-mediated Ca^{2+} transients ($n=3$, $P< 0.01$). In the GluN2B-KD study, NMDA (30, 100, 300 μM) application without antagonist showed no significant difference in Ca^{2+} response between GluN2B-KD and control ($n=6$). The GluN2B-KD significantly reversed the inhibition of amplitude of NMDAR-mediated Ca^{2+} response by 15 minutes incubation of 100μM ifenprodil ($n=4$, $P< 0.01$) but not in 15 minutes incubation of 3.3mM Agmatine.

Conclusions: APV and ifenprodil significantly attenuated the intracellular NMDARs-mediated Ca^{2+} signals, indicating that the NMDARs-mediated Ca^{2+} transients' assay is specific to NMDARs. Agmatine's concentration-dependent inhibition of NMDARs-stimulated Ca^{2+} transients suggests that Agmatine is an effective antagonist of NMDARs in the spinal cord dorsal horn, which is consistent with our electrophysiological and neuropharmacological research showing that Agmatine is an effective inhibitor of NMDARs in the spinal cord dorsal horn. GLuN2B-KD and controls had similar NMDAR-mediated Ca^{2+} transients, and GluN2B-KD reversed the attenuation of NMDARs-mediated Ca^{2+} transients by ifenprodil but did not with Agmatine. These results further defined the Agmatine's effect on NMDAR-mediated Ca^{2+} transients, which will help the development of agmatine-based chronic pain therapeutics.

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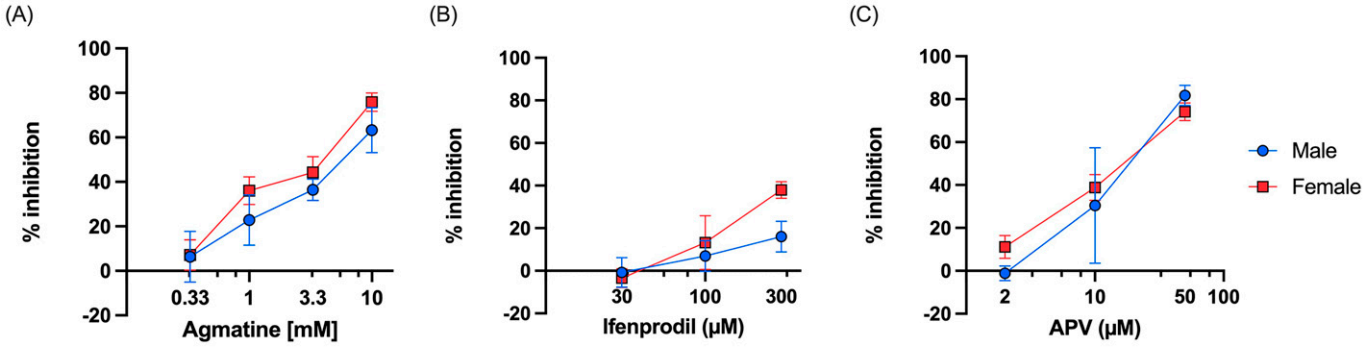

Figure 1. Agmatine (A), ifenprodil (B), and APV (C) concentration-dependently inhibit NMDAR-mediated Ca^{2+} transients.

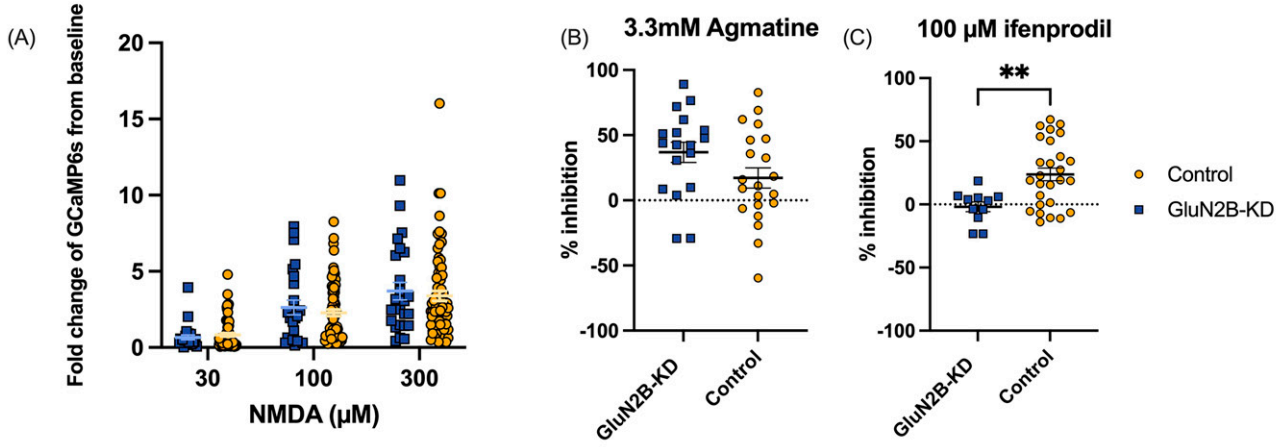

Figure 2. (A) GluN2B-containing NMDARs knock down (GluN2B-KD) animals show similar amplitude of NMDARs-mediated Ca^{2+} transients compared to control, and (B) GluN2B-KD reversed the NMDAR-mediated Ca^{2+} transients' attenuation by ifenprodil (n=4, P<0.01), (C) but not with Agmatine.