

## Methylglyoxal modulates pain via TRPA1/V1 and ROS in the spinal dorsal horn

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**INTRODUCTION:** Glycation is the non-enzymatic reaction of reducing sugars and related metabolites with proteins and amino acids, resulting in the production of Advanced Glycation End-product (AGEs) [1]. AGEs are known to be associated with various complications caused by diabetes and aging. Recently, Methylglyoxal (MGO), one of the precursors of AGEs, has been found to be associated with pain [2] [3].

However, the pain mechanism of MGO in the spinal dorsal horn neurons remains unclear. Therefore, we investigated the mechanism of action of MGO in spinal dorsal horn neurons using whole-cell patch-clamp recordings.

**METHODS:** All experimental procedures involving the use of animals were approved by the Ethics Committee on Animal Experiments, Wakayama Medical University, and were in accordance with the UK Animals (Scientific Procedures) Act, 1986, and associated guideline.

**Spinal cord preparation:** Male adult Sprague-Dawley rats (5-6 weeks of age) were deeply anesthetized with urethane, and then the lumbosacral spinal cord from L1 to S3 was removed and preserved in preoxygenated Krebs solution. After removing the pia and arachnoid membranes, the spinal cord was sliced using a micro slicer to a thickness of 650  $\mu$ m.

**Whole-cell patch-clamp recordings:** Whole-cell patch-clamp recordings were performed as described previously [4]. Recordings were made from substantia gelatinosa (SG) neurons using patch-pipette electrodes. Membrane potentials were maintained at -70mV in the voltage-clamp mode. In the experiment, MGO was perfused for 5 min and changes in the frequency and amplitude of sEPSC/miniature EPSC (mEPSC) were observed; the frequency and amplitude of sEPSC/mEPSC for 60 s before the start of MGO administration were used as controls and compared with the frequency and amplitude of sEPSC/mEPSC after MGO administration.

**Statistical analysis:** All numerical data are expressed as the mean  $\pm$  standard error of the mean (SEM). The paired Student's test was used to determine the statistically significant differences between means.

### RESULTS SECTION:

**Presynaptic effect of MGO in the spinal dorsal horn neurons:** The increase rates of the frequency and amplitude after administration of 10 mM MGO were 551.5% and 112.9% respectively (n=10). TTX, a voltage-gated Na<sup>+</sup> channel blocker, inhibits axonal conduction and allows the observation of mEPSC. In the presence of 1 $\mu$ M TTX, the increase rates of the frequency and amplitude after administration of MGO were 443.9% and 129.1%, respectively (n=5).

Subsequently, we examined the effect of MGO under the inhibition of AMPA/kainate receptors, which act primarily as glutamate receptors at VH = -70mV. In the presence of 20  $\mu$ M CNQX, as an AMPA/kainate receptor antagonist, no sEPSC were observed during MGO application (n=1). The result indicates that MGO increases the excitability of SG neurons by acting on pre-synapses and increasing glutamate release, which contributes to pain augmentation.

**TRPA1 and TRPV1 responsible for the MGO-induced increase in spontaneous glutamate release:** In the presence of 300 $\mu$ M ruthenium red, a non-selective antagonist of TRP channels, the rates of increase in frequency and amplitude after MGO administration were 92.2% and 99.4%, respectively (n=10).

Subsequently, the effects of MGO in the presence of 50 $\mu$ M HC-030031, a selective TRPA1 antagonist, were examined. There were no significant differences in the frequency or amplitude of sEPSC before and after perfusion with MGO. The rates of increase in frequency and amplitude after MGO administration in the presence of HC-030031 were 180.6% and 102.5%, respectively (n=9). The results suggest that TRPA1 participates in MGO reactivity in the spinal dorsal horn. Afterward, the association between TRPV1 and MGO-induced excitability of SG neurons was examined. In the presence of 10 $\mu$ M capsazepine, a TRPV1 antagonist, the mean frequency of sEPSC increased significantly (p < 0.05), whereas the amplitude of sEPSC did not increase significantly (n = 8). The rates of increase in frequency and amplitude after MGO administration were 234.9% and 103.3%, respectively. In the presence of capsazepine, MGO increased the frequency of sEPSC, but its potentiating effect tended to be suppressed when compared with that of MGO alone. The rates of increase in frequency and amplitude after MGO administration in the presence of HC-030031 and capsazepine were 107.5% and 100.1%, respectively (n=8). In the presence of HC030031 and capsazepine, the activity of MGO was suppressed completely in the dorsal horn of the spine.

**ROS associated with MGO-induced excitability of SG neuron:** MGO is metabolized to hydroimidazolone (MGO-H1), which binds to receptor for AGE (RAGE) [5][6]. This interaction between AGE and RAGE results in ROS production [7]. ROS has also been reported to act on TRPA1 and TRPV1 to promote excitatory synaptic transmission in spinal dorsal horn neurons [8]. Consequently, we hypothesized that MGO-induced ROS activates TRPA1 and TRPV1 in presynaptic terminals. To test the hypothesis, we investigated the effect of MGO in the presence of 1mM PBN, a non-selective ROS scavenger (n = 10). There were no significant differences between before and after perfusion with MGO in the presence of PBN.

**DISCUSSION:** Our study shows that MGO increases glutamate release and excites SG neurons by acting on TRPA1 and TRPV1 at the central end of primary sensory neurons. It is also revealed ROS involved in MGO-derived pain enhancing effect in spinal dorsal horn. The limitation is that we cannot determine where ROS is produced, which would be involved in the pain potentiation of MGO, and we also cannot determine whether ROS acts as a direct ligand for TRPA1 or TRPV1.

**SIGNIFICANCE/CLINICAL RELEVANCE:** (1-2 sentences): To the best of our knowledge, this is the first study to demonstrate that MGO induces pain through ROS production in the spinal dorsal horn. MGO, TRPA1, TRPV1, and ROS are potential therapeutic targets for glycation-related pain.

**REFERENCES:** Include references here. [1] Rabbani N +. *Kidney Int*, 2018. [2] Abdelkader H +. *Journal of Pharmacy and Pharmacology*, 2022. [3] Zhang Q +. *J Proteome Res*, 2009. [4] Nakatsuka T +. *Neuroscience*, 2000. [5] Thornalley P.J. +. *Ann N Y Acad Sci*, 2005. [6] Xue J +. *Biochemistry*, 2014. [7] Wautier M.P +. *Am J Physiol Endocrinol Metab*, 2001. [8] Nishio N +. *Neuroscience*, 2013.

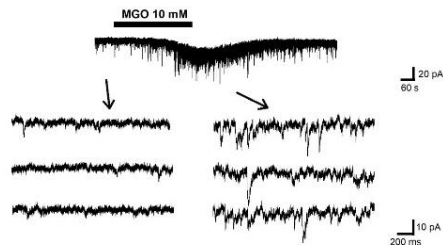


Fig A. Action of MGO on EPSC in SG neurons

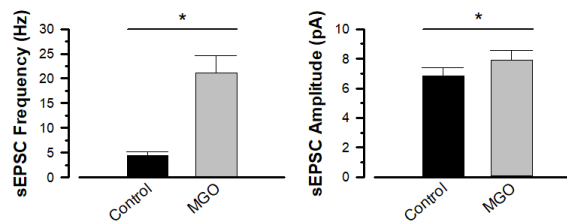


Fig B. Summary of sEPSC