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Pain control through Modulation of the Regulatory Mechanism of Purinergic Chemical Transmission by Manipulating Cl^- Balance

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Summary

Neuropathic pain is chronic pain associated with diabetes, cancer, viral infections, and hernias, and mediated by abnormalities in purinergic chemical transmission. Vesicular nucleotide transporter (VNUT) is responsible for vesicular ATP storage, and is essential for purinergic chemical transmission. We found that ATP transport activity is driven by membrane potential ($\Delta\psi$), and is allosterically regulated by Cl^-. In neuropathic pain, neuronal Cl^- concentrations increase with decreases in Cl^- exporter expression. Therefore, we hypothesized that a Cl^- imbalance would enhance vesicular ATP release, exacerbating neuropathic pain.

In this study, we aimed to demonstrate a new pathological mechanism of neuropathic pain mediated by Cl^- imbalance, and its effect on purinergic chemical transmission. Extensive screening using proteoliposomes containing only purified protein identified a physiological VNUT inhibitor with an IC_{50} of 67 nM, which acts as an allosteric modulator through competition with Cl^-. This inhibitor selectively inhibited vesicular ATP release from neurons. RT-qPCR indicated that mouse neurons expressed plasma membrane-type Cl^- importer and exporter, as well as VNUT. *In vivo*, we examined the effects of VNUT inhibitor on neuropathic pain using wild type and *VNUT*$^{-/-}$ mice. The VNUT inhibitor attenuated paclitaxel-induced neuropathic pain without affecting basal pain perception in wild type but not *VNUT*$^{-/-}$ mice. The inhibitor's analgesic effect against neuropathic pain was stronger than that of pregabalin or duloxetine, widely used analgesics for neuropathic pain. Cl^- importer inhibition attenuates paclitaxel-induced neuropathic pain, and combined inhibition of VNUT and Cl^- importer enhanced the analgesic effect against neuropathic pain. These results strongly suggest that VNUT senses Cl^- imbalance underlying neuropathy, and vesicular ATP release exacerbates neuropathic pain. As therapeutic analgesic agents with few side effects have yet to be developed, this VNUT inhibitor represents a new drug candidate for neuropathic pain that displays few side effects.