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Optical Inhibition of Neurons in Whole Rat Dorsal Root Ganglion after in vivo Intrathecal Viral Vector Induced Expression of the Optically Active Proton Pump Arch-T

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Introduction: Inhibition of neurons by selectively targeting sensory afferent neurons and maintaining motor neuron function would be of great interest. In addition, selective inhibition of afferent fiber subtypes would further allow control of nociceptive input to the spinal cord while potentially maintaining other sensory modalities. Optically active pumps have been shown to provide precise temporal control of neuronal inhibition with a millisecond time window (1). We hypothesized that intrathecal viral vector delivery of the optically active proton pump Arch-t could be accomplished resulting in neuronal transduction and expression and that peripheral afferent neurons could be inhibited using optical activation of Arch-t.

Methods: After Animal care and use committee approval, male Sprague-Dawley rats were placed under general anesthesia and prepped with Betadine. A 30 g needle was placed into the intrathecal space at the L3-4 vertebral space. After a tail flick was elicited as previously described (2), 10 μ L of replication deficient self complementary AAV8 vector containing Arch-t with a GFP tag was injected (3). Three to four weeks after injection, 4 animals were anesthetized and perfused with 4% paraformaldehyde and tissue was cryoprotected. Dorsal root ganglia (DRG) at L5 and 6 were sectioned and imaged using a Nikon fluorescent microscope. Four other animals were placed under general anesthesia and the L5 and 6 DRG were removed and placed in ice cold artificial CSF. Whole DRG were mounted on an inverted microscope and whole cell intracellular recordings were obtained in current clamp at 37 degrees as previously described (4). Resting membrane potential (RMP) in millivolts (mV) and rheobase (RHEO) in nanoamperes (nA) were measured before, after 2 and 5 minutes light exposure and after the light was off for 5 minutes. Data are presented as means with standard deviation. The paired t-test was used to test for statistical significance or Chi square.

Results: Following intrathecal injection, there was a time dependent increase in expression of ARCH-t/GFP in the DRG cells. Transduction only occurred in sensory afferents and not in the motor neurons nor in the spinal cord. Expression was noted throughout the axon, dendrite and soma of the afferent including projections to the dorsal horn of the spinal cord and the skin. RMP and RHEO were measured in twelve cells. The average RMP was -63.4 ± 8.3 mV and the RHEO was 1.98 ± 0.64 nA. After 2 minutes exposure to 480-550 nm light, the RMP became hyperpolarized to -75 ± 7.6 mV ($p < 0.05$) (figure 3), while the RHEO increased to 3.27 ± 1.17 nA ($p < 0.05$). After 5 minutes exposure, no action potential (AP) could be generated in 4/12 cells ($p < 0.05$ compared to 0 at 2 minutes). After the light was turned off, the RMP and the RHEO returned to baseline. Even cells that could not generate an AP in the presence of the light, returned to normal AP generation 5 minutes after the light exposure was terminated.

Discussion: This is the first report of optical inhibition of sensory afferent neurons from the DRG after in vivo intrathecal administration of Arch-T using a viral vector. The lack of expression of the channel in motor neurons is beneficial, but the etiology of the specificity is unclear. Further studies will focus on targeting specific fiber subtypes of afferent sensory neurons using selective promoters. We will also focus on establishing the ability to inhibit the DRG neurons in vivo. These techniques will be valuable for exploring the role of afferent activity in pain circuits and may lead to therapeutic interventions utilizing light, possibly patient controlled, for spatial and temporal control of afferent nociceptive input to the spinal cord to control pain.

References

1. Nature. 2010;463:98-102.
2. Anesthesiology 2004;101:1031-5.
3. <http://www.syntheticneurobiology.org>

4. Anesthesiology 2008;109:111-7.

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