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GENERATING TRPV1 AND GCAMP6 DOUBLE-TRANSGENIC MICE TO STUDY MUSCLE PAIN

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Background

The Cre/loxP recombination system is a site-specific genetic engineering technique used when selecting for the deletion or activation of a specific gene [1,2]. By using this technique, the Light lab is interested in creating mice that have endogenous calcium sensors (GCaMP6) with promoters (such as TRPV1) that allow expression in the Group III/IV muscle innervating sensory neurons. This novel mouse strain will allow us to record the metabolite responses of the sensory nerves both *in vivo* and *in vitro*. TRPV1 is a membrane receptor that is almost exclusively expressed in a subset of nociceptors in the periphery [3]. Both TRPV1-Cre and GCaMP6 mouse strains have become available recently, which makes our goal feasible. The TRPV1-Cre mouse has a gene for a recombinase enzyme engineered in what's referred to as "Cre". The second mutant mouse crossed, carried a stop codon and a GCaMP6 gene engineered into the genome. This stop codon is floxed by two loxP sequences (sites for recombinase), followed by the GCaMP6 gene to be activated. The primary role of Cre in this system is to catalyze recombination. It accomplishes this by splicing the sequence between each loxP site. The floxed stop codon in front of GCaMP6 will be deleted in cells expressing Cre (and TRPV1), allowing the expression of GCaMP6—a calcium activated green fluorescent protein—in these neurons. Thus, GCaMP6 is only synthetized in the sensory neurons that express TRPV1 and Cre.

Research Question

Using the Cre/loxP recombination system, we are interested in determining (1) the outcomes of the mating, and (2) whether or not GCaMP6 in the DRG neurons of the new strain coincides with the expression of TRPV1 receptors. In other words, do the green fluorescent cells also respond to capsaicin, a specific agonist for TRPV1?

Methods

Homozygous male TRPV1-Cre mouse and hemizygous female GCaMP6 mouse were bred to attain offspring that express GCaMP6 only in TRPV1-expressing cells. To genotype the pups, tissue samples (ear punch under isoflurane anesthesia) were collected, and incubated at 95°C in lysis buffer for one hour. A real-time PCR reaction was performed using appropriate primes for the target genes, to amplify target DNA sequences in samples. Products were then used to run DNA electrophoresis (1.5% agarose in TBE buffer). Bands were identified by comparison to a 100 base pair ladder. Once genotyping was complete and verified, 3 weeks old GCaMP6/TRPV1-Cre double positive pups were selected for dissection to obtain L2-L6 lumbar Dorsal Root Ganglia (DRG). These lumbar DRGs received sensory input from the hind limbs. To identify

sensory neurons that specifically innervate the skeletal muscle, a red fluorescent dye (DiI) was injected in the hind limb muscles on day 7-9 under anesthesia in ice water. The dye can be retrogradely transported to the neuronal body in the DRGs. The DRGs were dissociated for primary neuronal culture on 24-well polystyrene plates with a nutrient medium and were allowed to develop in a 37 °C, 5% CO_2 incubator for approximately 12-16 hours. Calcium imaging was then used to observe the cultures and record fluorescent signals in the cells in response to application of 80nM capsaicin. Under the fluorescence microscope, DiI showed red fluorescence, while GCaMP6 expressing neurons showed green fluorescent signal. Cells that were double labeled with DiI and GCaMP6 appeared to be yellow in color.

Results

Genotyping results showed that GCaMP6 and Cre were both present in approximately 50% of the offspring. Calcium imaging study showed that an increase in green fluorescence (GCaMP6) was observed after adding capsaicin. The increase was due to the influx of calcium ions through TRPV1 ion channels opened in these cells.

Summary

The Cre/loxP recombination system is an effective tool for selective expression of GCaMP6 gene in TRPV1-expressing primary sensory neurons. GCaMP6 showed bright signals and an increase in fluorescence was observed in cells that express TRPV1 (manifested by responding to capsaicin). The novel TRPV1/GCaMP6 double transgenic mice would be useful for the muscle pain study. However, although cultured cells showed strong signals, future study is necessary to determine whether or not the fluorescence is strong enough for *in vivo* recording, especially in nerve endings.

References

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