TRPM8-neurons mediate the inhibition of itch at cold temperatures

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Introduction

Many forms of acute itch (pruritus) are unresponsive to anti-histamine treatment. The anti-malarial drug Chloroquine is one such histamine-independent pruritogen. Previous work in the McKemy laboratory has shown that cooling the affected area can inhibit Chloroquine-induced itch. The transient receptor potential ion channel TRPM8 is a molecular sensor of cold stimuli. Therefore, the primary sensory neurons expressing TRPM8 are required for detecting pleasant as well as noxious cold sensations. My hypothesis is that cooling-induced anti-pruritus is also mediated by TRPM8-expressing neurons. To test this hypothesis, I compared the effects of cooling on itch-induced by a subcutaneous injection of Chloroquine (200µg) in wild-type and TRPM8-neuron ablated C57BL/6J mice. My results indicate that TRPM8-neurons are required to inhibit Chloroquine-induced acute itch at 10°C. Cold sensing neurons can therefore be targeted with strong pharmacological agonists of TRPM8 to relieve the side effects of Chloroquine.

Central Hypothesis

Cooling inhibits itch in a TRPM8-neuron dependent manner
1. Cooling inhibits itch?
2. Does cooling mediated inhibition of itch need TRPM8-neurons?

Methods

1. Mouse models:
   - The McKemy lab has developed a mouse line in which TRPM8 neurons express a highly sensitive epithelial toxin receptor (DTR). By injecting small amounts of the toxin (DTX), TRPM8-expressing neurons can be specifically killed (Knowlton et al., 2013).
   - I used these ablated mice to determine the role of TRPM8-neurons in inhibiting Chloroquine-induced itch.

2. Experimental paradigm
   - My hypothesis is that cooling inhibits Chloroquine-induced itch by activating TRPM8-neurons. Therefore, TRPM8-neuron ablated mice injected with Chloroquine should not show reduced itch behaviors at cold temperatures.

3. Method:
   - 200µg of Chloroquine was injected into one hindpaw of either wild-type or TRPM8-neuron ablated animals.
   - Animals were placed on a plate set to 24°C, 20°C or 10°C.
   - Duration of licking and biting of the injected paw was measured over 30 minutes post-injection.

Results

1. The wild-type animals placed at 20°C for the first 15 minutes of the experiment licked only for 13.7 ± 6.2 seconds. (red bars)
   - This is significantly less than the behavior displayed by mice at 24°C in the same time (95.35 ± 16.1 seconds, p<0.05, blue bars).
   - Once the cold stimulus was removed and the plate temperature was raised to 24°C in the last 15 minutes, there was an increase in the itch behavior seen in the previously unresponsive mice (106.8±16.8 seconds, red bars).
2. The mice placed at 24°C throughout the experiment show reduced itch in the last 15 minutes (27.69±13.9 seconds), perhaps due to the inhibition caused by licking and biting shown during the first 15 minutes.

Future Directions

1. Increase sample size of ablated animals injected with 200µg of Chloroquine at 20°C.
2. Determine if ablated animals at 24°C itch as much as WT animals.
3. Determine if cooling inhibits acute itch induced by other pruritogens, eg. Histamine.
4. Determine if cooling inhibits chronic itch (dry skin).
5. IT RPM8-neurons are required in the inhibition of acute and chronic itch by cooling.
6. Investigate the effects of chemical cooling on itch. (eg. Menthol).

Conclusion

- My results indicate that TRPM8-neurons are required to mediate the inhibition of Chloroquine-induced itch at cold temperatures (especially at 10°C).
- These experiments have not only confirmed the role of TRPM8-neurons in cooling-mediated itch relief, they have also revealed a novel anti-pruritic pathway.
- This TRPM8-dependent sensory pathway can be pharmacologically targeted to improve treatment for Chloroquine-induced itch.

References

1. McKemy, David D. Chapter 13 TRPM8: The Cold and Menthol Receptor. 2007 PubMed:21204488

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