Supporting Information

G9a mediates diminished expression of cannabinoid CB_1 receptors in primary sensory neurons in neuropathic pain

Yi Luo, Jixiang Zhang, Lin Chen, Shao-Rui Chen, Hong Chen, Guangfen Zhang, Hui-Lin Pan

Validation of CB_1R Antibodies Using CB_1R cKO Mice and Immunoblotting

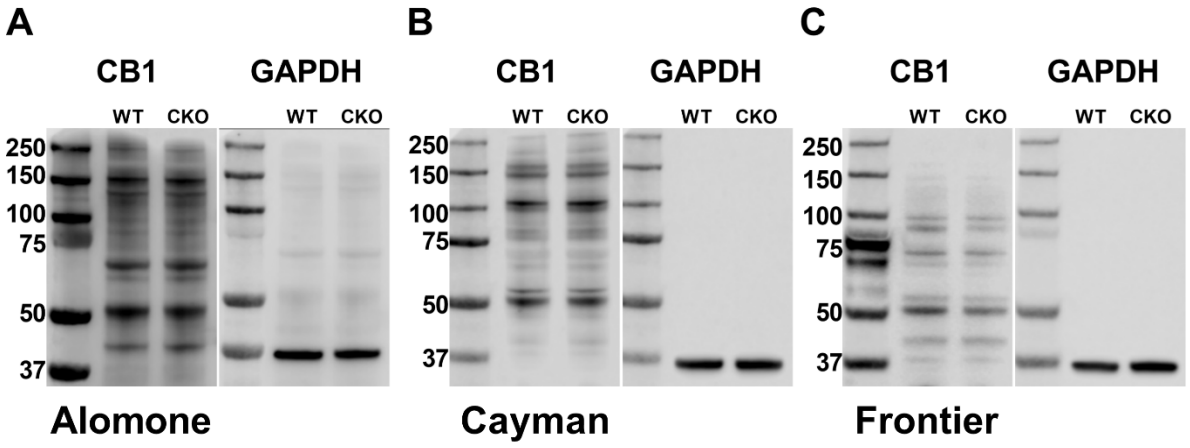
Methods

CB_1R (encoded by the *Cnr1* gene) conditional knockout (cKO) mice were generated by crossing female *Cnr1*$^{flox/flox}$ mice with male *Advillin*$^{Cre/+}$ mice. *Cnr1*$^{flox/flox}$ mice were produced as described previously (1). CB_1R cKO mice were identified via genotyping, and a profound reduction in the mRNA level of CB_1Rs in the DRG was confirmed using quantitative PCR.

Lumbar DRG tissues were obtained from adult wild-type and CB_1R cKO mice, dissected, and homogenized in cold radioimmunoprecipitation assay lysis buffer containing 1% protease inhibitor cocktail (Sigma-Aldrich). After centrifugation (13,000 g for 20 min at 4 ℃), the supernatants of the samples were collected. Equal amounts (30 μg) of proteins were separated by electrophoresis using a 4–12% gradient sodium dodecyl sulfate-polyacrylamide gel, transferred to a polyvinylidene fluoride membrane, and incubated in the blocking solution (5% nonfat dry milk) for 1 h. The following primary antibodies were used: rabbit anti-CB_1R (1:600; #ACR-001, Alomone Labs, Jerusalem, Israel), rabbit anti-CB_1R (1:1000; #10006590, Cayman Chemical, Ann Arbor, MI), rabbit anti-CB_1R (1:2000; #AB_2571593, Frontier Institute Co., Hokkaido, Japan), and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:2,000; #5174, Cell Signaling Technology, Danvers, MA). The specificity of these anti-CB_1R antibodies has been reported previously (2-4).

Results

Figure S1 below shows representative blotting images of the protein bands detected by the three CB_1R antibodies in DRG tissues obtained from wild-type and CB_1R cKO mice. The GAPDH protein band on the same gel was used as a loading control, and molecular weight markers are shown on the left of the blotting images. The three CB_1R antibodies from Alomone Labs (A), Cayman Chemical (B), and Frontier Institute Co. (C) detected similar protein bands in the DRG tissues obtained from wild-type and CB_1R cKO mice. Immunoblotting was repeated for each antibody using separate DRG samples at least three times. These findings indicate that the three antibodies tested are not specific for CB_1R proteins.



Supplemental references

1. Mani, B. K., Castorena, C. M., Vianna, C. R., Lee, C. E., Metzger, N. P., Vijayaraghavan, P., Osborne-Lawrence, S., Elmquist, J. K., and Zigman, J. M. (2019) Combined Loss of Ghrelin Receptor and Cannabinoid CB1 Receptor in Mice Decreases Survival but does not Additively Reduce Body Weight or Eating. *Neuroscience*, doi: 10.1016/j.neuroscience.2019.1009.1005
2. Hajkova, A., Techlovska, S., Dvorakova, M., Chambers, J. N., Kumpost, J., Hubalkova, P., Prezeau, L., and Blahos, J. (2016) SGIP1 alters internalization and modulates signaling of activated cannabinoid receptor 1 in a biased manner. *Neuropharmacology* **107**, 201-214
3. Gonzalez-Islas, C., Garcia-Bereguiain, M. A., and Wenner, P. (2012) Tonic and transient endocannabinoid regulation of AMPAergic miniature postsynaptic currents and homeostatic plasticity in embryonic motor networks. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **32**, 13597-13607
4. Agarwal, N., Pacher, P., Tegeder, I., Amaya, F., Constantin, C. E., Brenner, G. J., Rubino, T., Michalski, C. W., Marsicano, G., Monory, K., Mackie, K., Marian, C., Batkai, S., Parolaro, D., Fischer, M. J., Reeh, P., Kunos, G., Kress, M., Lutz, B., Woolf, C. J., and Kuner, R. (2007) Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nature neuroscience* **10**, 870-879