Cannabinoids increase synaptic vesicle filling at the neuromuscular junction

M. Morsch,[1,2] D.A. Protti,[2] D. Cheng,[3] F. Braet,[3,4] R.S. Chung,[1] S.W. Reddel[5] and W.D. Phillips ,[2] [1]Department of Biomedical Sciences, Macquarie University, NSW 2109, Australia, [2]Discipline of Physiology & Bosch Institute, The University of Sydney, NSW 2006, Australia, [3]Discipline of Anatomy and Histology, School of Medical Sciences, The University of Sydney, NSW 2006, Australia, [4]Australian Centre for Microscopy & Microanalysis (ACMM), The University of Sydney, NSW 2006, Australia and [5]Departments of Molecular Medicine & Neurology, Concord Clinical School, The University of Sydney, NSW 2006, Australia.

In the brain endocannabinoids mediate negative feedback regulation of quantal transmitter release when postsynaptic neurons become depolarized. We have begun to investigate the effects of the cannabinoid receptor agonist WIN 55,212 upon quantal synaptic transmission at the mouse neuromuscular junction (Morsch *et al.*, 2018). *Ex vivo* phrenic nerve-hemidiaphragm preparations from adult C57BL6J mice were impaled close to the neuromuscular junction with a sharp capillary microelectrode filled with 3M KCl. Endplate potentials, corrected for non-linear summation were recorded in the presence of μ-conotoxin to block action potentials. Addition of 10μM WIN 55,212 to the bathing solution caused an acute 1.4 fold increase in the mean EPP amplitude, which could be fully explained by a parallel increase in the spontaneous miniature EPP amplitude. Similar increases in mEPP amplitude were produced by the endocannabinoid anandamide and by a specific inhibitor of fatty-acid amide hydrolase, URB597, which blocks degradation of endogenous anandamide. The effects of WIN 55,212 were occluded by inverse agonists of the CB1 receptor, AM251, and the CB2 receptor, AM630, to the bath solution. The increase in quantal amplitude could be explained by a WIN 55,212-induced increase in synaptic vesicle diameter/capacity. These results suggest that cannabinoids may act presynaptically to increase synaptic vesicle filling. We propose that endocannabinoids might help regulate synaptic potentials to maintain control of the muscle during sustained muscle use, when quantal number becomes limiting.

Morsch M, Protti DA, Cheng D, Braet F, Chung RS, Reddel SW, Phillips WD. (2018). Cannabinoid-induced increase of quantal size and enhanced neuromuscular transmission. *Sci Rep* **8,** 4685. doi: 10.1038/s41598-018-22888-4