TRPV1 Channels Mediate Linoleic acid-evoked Calcium Responses In Adult DRG Neurones

Mohammad Alsalem, Paul Millns, victoria chapman, David Kendall

School of Biomedical Sciences, University of nottingham, Medical School, Queen’s Medical Centre, NG7 2UH, Nottingham, Nottinghamshire, United Kingdom

Recent work has shown that 9- and 13-HODE, which are metabolites of linoleic acid, activate TRPV1 channels and may contribute to the activation of this receptor by noxious thermal heat (Patwardhan et al., 2010). The involvement of lipoxygenases in the generation of these endogenous ligands for TRPV1 has been described (Patwardhan et al., 2010). The aim of the present study was to evaluate further the contribution of TRPV1, and lipoxygenase metabolism, to the calcium responses of adult rat dorsal root ganglia (DRG) neurones following exposure to linoleic acid.

Small diameter DRG neurones (<30µm) were dissected from male Sprague-Dawley rats (180-200g) and loaded with the calcium sensitive ratiometric dye Fura 2AM. Capsaicin (100nM) and linoleic acid (1mM)-evoked changes in [Ca\(^{2+}\)] were measured in single neurones using an Improvision imaging system. Intracellular Ca\(^{2+}\) concentration [Ca\(^{2+}\)] was estimated as ratios of peak fluorescence intensities (measured at 500nm) at excitation wavelengths of 340 and 380nm respectively. The effects of pre-exposure to capsazepine (10µM) or the non-selective lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA, 100nM) on linoleic acid-evoked calcium responses were determined. Finally, the effects of pre-exposure to NDGA (100nM) on capsaicin-evoked calcium responses were determined.

Linoleic acid produced a robust increase (0.59±0.03) in [Ca\(^{2+}\)] in DRG neurones, with an onset time of approximately 5 minutes post-exposure. Capsaicin also produced a robust increase (0.57±0.02) in [Ca\(^{2+}\)] in DRG neurones, but had a more rapid onset time. 60% of DRG neurones (n=438 cells) responded to both linoleic acid and capsaicin and 9% of DRG neurones only responded to linoleic acid. Pre-exposure to capsazepine significantly decreased the number of cells responding to linoleic acid (9% responsive, n=295 cells). Of those cells which responded to linoleic acid, the mean response was 0.32±0.04. The number of cells responding to linoleic acid was attenuated by the lipoxygenase inhibitor NDGA (30% reduction). Linoleic acid-evoked increases in [Ca\(^{2+}\)] were decreased by NDGA to 60±3% of the linoleic acid response in the presence of vehicle. Importantly, neither the capsaicin-evoked calcium responses, nor the number of cells responding to capsaicin, were altered by pre-exposure to NDGA, compared to vehicle.

In summary, linoleic acid produces slow, but robust, increases in [Ca\(^{2+}\)] in DRG neurones, which are mediated, at least in part, by TRPV1. The onset time of the response to linoleic acid, the ability of NDGA to reduce the number of cells, and the magnitude of the response to, linoleic acid, support the proposal that linoleic acid is metabolised to an active TRPV1 ligand. Our data are consistent with the report that 9- and 13-HODE, generated from linoleic acid, directly activate TRPV1 in vivo and in trigeminal neurones (Patwardhan et al., 2010). Future work will investigate the direct effects of 9- and 13-HODE on [Ca\(^{2+}\)] responses in adult DRG neurones.