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Determining The Signalling Pathway Mediating Endocannabinoid Inhibition Of Airway Sensory Nerve Depolarisation And The Cough Reflex

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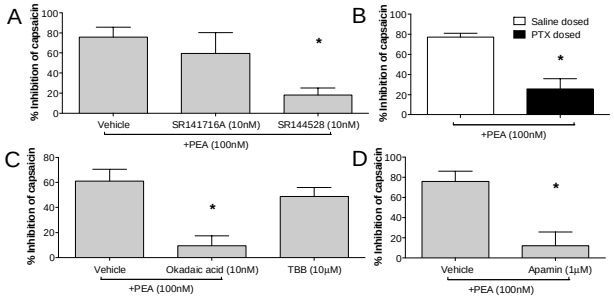
Cough is an essential defensive reflex event, however excessive/chronic cough can be a problematic symptom of respiratory diseases, for which no effective and safe medications are available[1]. Fatty acid amide hydrolase (FAAH) is an enzyme that catalyses the hydrolysis of amidated signalling lipids. FAAH inhibition elevates endocannabinoid levels (including palmitoylethanolamide, anandamide, oleoylethanolamide and linoleoylethanolamide: PEA, AEA, OEA, LEA), causing inhibition of citric-acid evoked cough in guinea pigs, and PEA inhibits sensory nerve depolarisation[2]. Here we investigate the signalling pathway involved in endocannabinoid inhibition of sensory nerve/neuron activity.

Isolated vagal nerves or neurons were dissected from male Dunkin-Hartley guinea pigs (300-500g, Harlan) or human lung (unsuitable for transplant)[3,4]. PEA (1-1000nM) inhibited capsaicin-induced depolarisation of guinea pig vagus nerve (max 84.2±6.7% n=4). PEA also inhibited capsaicin-induced calcium flux in guinea pig airway-terminating jugular neurons (62.7±4.9% N=3). A CB_2 (SR144528) but not CB_1 (SR141716A;) antagonist (10nM) reversed PEA inhibition in guinea pig and human vagus depolarisation (fig 1a). Similarly the endocannabinoids AEA, OEA and LEA (100nM) also inhibited depolarisation (~70%) of guinea pig vagus in a CB_2 dependent manner. Furthermore, PEA no longer inhibited depolarisation in guinea pigs dosed 3 days prior (25µg i.p.) with pertussis toxin (fig 1b), indicating a role for the G-protein α_i subunit. Apamin (1µM: SK_{Ca} blocker) reversed PEA inhibition of guinea pig and human vagus depolarisation (fig 1c). Furthermore, PEA inhibited depolarisation to the TRPA1 agonist acrolein (300µM;79.7±6.9%, n=3 $p<0.05$ paired



t test) and the TRPV4 agonist GSK1016790a (300nM;64.2±10.7%, n=3 $p<0.05$ paired t test) indicating a general suppression of depolarisation concurrent with enhanced SK_{Ca} activity. PP2A and CK2 increase and decrease the calcium sensitivity of SK_{Ca}[5]. Okadaic acid (10nM; PP2A inhibitor) blocked PEA inhibition of guinea pig vagus depolarisation, and additionally, TBB (10µM:CK2 inhibitor) itself inhibited depolarisation (fig 1d).

Together this data suggests the endocannabinoid PEA inhibits sensory nerve activation in guinea pig and human vagus nerve via a $CB_2/G_i/PP2A/SK_{Ca}$ pathway. Furthermore, this data implies that inhibiting FAAH/elevating endocannabinoids may be beneficial in conditions driven by excessive peripheral sensory nerve activation including chronic cough.

NB – drug vehicle 0.1% DMSO unless stated, sub-maximal conc of PEA (100nM) or capsaicin (1μM) used unless stated. Groups compared to vehicle controls using Mann-Whitney U or Kruskall-Wallis w/Dunns post-hoc, $p<0.05$ considered significant.

1. Nasra J and Belvisi MG (2009). *Pharmacol Ther* **124:** 354–375
2. Adcock, J. J. *et al. (2011).Proc Br Pharmacol Soc.* **9:** A025p
3. Belvisi, M. G. *et al. (2009). Br J Pharmacol* **155:** 547–557
4. Dubuis ED *et al.* (2013). *Curr. Protoc. Pharmacol.* **62:** Unit 12.15
5. Adelman JP *et al.* (2012). *Annu. Rev. Physiol.* **74:** 245–69