ETHANOL SENSITISES TRPV1-MEDIATED COUGH IN GUINEA PIGS BY A PROTEIN KINASE C-DEPENDENT PATHWAY

Gatti Raffaele[1], Andre Eunice[1], Campi Barbara[1], Geppetti Pierangelo[1, 2] and Trevisani Marcello[1]

[1]Interdisciplinary Centre of Excellence for the Study of Inflammation (ICSI), University of Ferrara, Ferrara, Italy.
[2]Department of Critical Care Medicine and Surgery, University of Florence, Florence, Italy.

Background Alcoholic beverages are known to affect a wide range of pathologies including rhinitis, itching, headache, asthma and cough. Ethanol has been shown to sensitise the transient receptor potential vanilloid 1 (TRPV1) (Trevisani et al, 2002) to a large variety of stimuli, by mechanism largely dependent on protein kinase C (PKC). Here, we set out to investigate whether ethanol sensitizes TRPV1-dependent excitation of isolated sensory neurons and TRPV1-dependent cough in guinea pigs and weather a PKC-dependent mecahnism was involved in the sensitisation. **Methods and Results** Cough was induced by aerosolised citric acid (CA, 0.25 M, 10 min: 11.5 ± 1.0 number of coughs; $P<0.05$ vs. hysotonic saline, 0.9%) and by the ultrapotent TRPV1 agonist, resiniferatoxin (RTX, 0.5 µM, 10 min: 10.9 ± 0.8 number of coughs; $P<0.05$ vs. hysotonic saline, 0.9%). Exposure to an aerosol of 3% EtOH for 10 min prior to the cough challenge did not produce any coughs *per se* but significantly enhanced the number of coughs induced by CA and RTX (18.5 ± 2.4 and 14.8 ± 0.7, respectively, $P<0.05$). In contrast, aerosolised EtOH (3%) was unable to enhance the number of coughs induced by hypertonic saline (7%), a tussive agent that acts through a TRPV1-independent mechanism. To investigate the mechanism by which ethanol enhances TRPV1-mediated cough response, we firstly used the protein kinase C activators, TPA. TPA (10 µM), as previosly shown, significantly enhances RTX-induced cough, an effect completely prevented by the PKC inhibitor, GFX (1 µM). Furthermore, aerosolised GFX pratically abolished the cough exacerbation produced by pre-exposure to ethanol 3%. Similarly, EtOH- and TPA-induced exacerbation of RTX-induced calcium (Ca^{2+}) mobilisation was significantly prevented by GFX. ***Discussion and Conclusions*** The present data shows that EtOH selectively exaggerates, *via* a PKC-dependent pathway, the Ca^{2+} response in cultured sensory neurons and cough evoked by TRPV1 stimulation in guinea pigs. EtOH selectively exaggerates the CA- and RTX-, but not hypertonic saline, induced cough in guinea pigs. Potentiation by EtOH of responses mediated by sensory neurons *in vitro* and *in vivo* may be due to TRPV1 sensitization. These findings suggest the alcohol-evoked exaggeration of sensory input and reflex responses may play a role in the clinical manifestation in different respiratory conditions, including chronic cough and asthma.