Nociceptin/Orphanin FQ Inhibits Cough in the Guinea Pig and Cat by Activation of NOP_1 Receptors. L.E. Parra, R.L. McLeod, D.C. Bolser[1], J.C. Mutter, D.B. Tulshian[2], R.W. Egan and J.A. Hey. Allergy, Schering-Plough Research Institute, NJ USA; [2]Chemistry, Schering-Plough Research Institute, NJ USA; [1]University of Florida, FL USA.

Recently, a new opioid-like receptor, NOP_1, and its endogenous ligand, nociceptin/orphanin FQ (NC/FQ), have been discovered. Drugs that activate opiate receptors produce antitussive activity and therefore, we studied the antitussive effects of NOP_1 receptor activation with NC/FQ in models of irritant and mechanically-induced cough. In guinea pigs, NC/FQ (10, 30, and 90 μg) given into the CNS by intracerebroventricular (i.c.v.) route dose-dependently inhibited capsaicin-induced cough by 23 ± 9, 29 ± 15 and 53 ± 3%, respectively. The antitussive activity of NC/FQ (90 μg, i.c.v.) was blocked by the NOP_1 peptide antagonist, [Phe$^1\psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH2 (180 μg, i.c.v.) and the nonpeptide antagonist, J113397 (10 mg/kg, i.p.) but not by the μ opioid antagonist, naltrexone (3 mg/kg, i.p.). Furthermore, i.v. NC/FQ (1.0 and 3.0 mg/kg) also inhibited cough by 23 ± 11 and 40 ± 12%, respectively. In cats, i.v. NC/FQ (0.01-3.0 mg/kg) produced a dose-dependent inhibition of mechanically-induced cough. This antitussive effect was blocked by pretreatment with J113397 (0.1 mg/kg, i.v.). These findings indicate the NOP_1 activation with NC/FQ inhibits cough when administered by central or peripheral routes, and may represent a novel therapeutic approach for the treatment of cough.

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ANANDAMIDE INDUCED DEPOLARIZATION OF GUINEA-PIG ISOLATE VAGUS NERVE M Kagaya, CP Page & D Spina[1] Sackler Institute of Pulmona Pharmacology, GKT School of Biomedical Sciences, King's College London, Lond SE1 9RT, [1]GKT School of Medicine, King's College London, London SE5 9PJ, UK

We have reported that the endogenous cannabinoid receptor agonist, anandami may be a partial agonist of the VR1 on guinea-pig isolated bronchus (Tucker et a 2001 Br. J. Pharmacol. 132, 1127-1135). In the present study, we further investigate the effect of anandamide against VR1 by electrophysiological technique on guinea-p vagus nerve. The de-sheathed vagus nerve was placed in a gap junction and perfuse with HEPES buffer solution. Depolarization to agonist was recorded as changes voltage to a reference electrode before and following 10 sec perfusion with agonis Both capsaicin (pD_2 = 5.32 ± 0.12, Emax, %40mM KCl: 24.17 ± 1.78, n = 4) an anandamide (pD_2 = 5.56 ± 0.04, Emax: 16.30 ± 1.46, n = 4) produced a concentratio dependent depolarization response. In the presence of the VR1 selective antagonis capsazepine (CRZ, 10 μM), the depolarisation to capsaicin (19.54 ± 1.28 vs CP 10.29 ± 1.62, n = 5, $p < 0.01$) and anandamide (14.19 ± 1.30 vs CPZ, 7.89 ± 0.63, n 7, $p < 0.01$) was significantly inhibited. In contrast, this response was unaltered by th CB1 selective antagonist SR141716A (1 μM) (capsaicin: 17.98 ± 1.90 vs SR141716 17.22 ± 2.07, n = 4, $p > 0.05$) (anandamide: 12.54 ± 2.52 vs SR141716A, 12.05 1.82, n = 4, $p > 0.05$) and CB2 selective antagonist, SR144528 (1 μM) (capsaicir 18.06 ± 1.17 vs SR144528 16.47 ± 1.01, n = 4, $p > 0.05$) (anandamide: 12.54 ± 1.8 vs SR144528, 11.80 ± 1.59, n = 4, $p > 0.05$). The CB agonist, CP55940 (10 μM failed to depolarize the nerve (2.30 ± 0.53, n = 6). These results demonstrate tha anandamide evokes depolarization, secondary to activation of vanilloid receptors o guinea-pig vagus nerve.

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THE EFFECT OF KINASE INHIBITOR K252a ON SUBSTANCE P (SP) INNERVATION FOLLOWING TOLUENE DIISOCYANATE (TDI) EXPOSURE. E.R. Sikora and R.D. Dey. Department of Neurobiology and Anatomy, West Virginia University, Morgantown, WV, 26506

TDI causes rhinitis, nasal irritation and increased synthesis and release of SP from airway sensory neurons. The peripheral signaling mechanism responsible for enhanced SP production remains unclear but may involve nerve growth factor (NGF), which also increases following irritant inhalation. NGF binds tyrosine kinase A (trkA) receptors located on sensory nerve terminals. Activation of trkA receptors initiates kinase signaling cascades, which ultimately may increase SP synthesis. In this study, the effect of K252a, a protein kinase inhibitor, nasally instilled prior to TDI was evaluated using SP nerve fiber density (NFD) measurements in the nasal epithelium. Both nasal cavities of adult Sprague-Dawley rats were instilled with either 8μl of K252a (100 μg/ml) or DMSO (control). Two hours later the nasal cavities were again instilled with 5μl of either 10% TDI or ethyl acetate (control). Nasal lavages and removal of nasal mucosa were performed 24hrs after TDI instillation (n≥3/group). K252a/TDI rats had significantly less SP NFD in the nasal epithelium (.20±.02) and less neutrophils in the nasal lavages (6.0±4.5) than the DMSO/TDI group (.52±.03 and 80.8±3.4 respectively). These findings suggest that TDI-induced SP upregulation in airway neurons is potentially mediated by kinase pathways activated by NGF bound trkA receptors.

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OZONE EXPOSURE DURING POSTNATAL DAYS (PD) 2-6 ENHANCES SUBSTANCE P (SP) -INNERVATION IN AIRWAY SMOOTH MUSCLE OF RAT PUPS R.D. Dey, B.E. Satterfield and T Mulvey. Department of Neurobiology and Anatomy, West Virginia University, Morgantown, WV 26506.

Irritant exposure during early postnatal life may affect airway responses to subsequent exposures. In previous studies, SP innervation of intrapulmonary airway smooth muscle in PD 29 rat pups was increased after a single exposure to 2 ppm ozone on PD 4 and re-exposure on PD 28. No affect was observed if the first exposure occurred on PD 21, suggesting greater susceptibility to ozone during the earlier postnatal period. In the present study, rats were exposed to 2 ppm ozone for 1 hr on PD 2-6 or PD 19-23, re-exposed on PD 28 and evaluated on PD 29. SP innervation of smooth muscle in extrapulmonary and intrapulmonary bronchi was increased relative to controls in the PD 2-6 exposure group but not in the PD 19-23 group. Ozone exposure on PD 2-6 without re-exposed on PD 28 was not different from controls. Neutrophil counts in lavage fluid from the PD 2-6 group were not different from controls suggesting the absence of inflammation on PD 29. The findings confirm that postnatal exposure to ozone on PD 2-6, but not PD 19-23, with re-exposure on PD 28 enhances SP innervation in both intrapulmonary and extrapulmonary smooth muscle and occurs in the absence of inflammation.

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OZONE-ENHANCED AIRWAY HYPERRESPONSIVENESS INVOLVES INTRINSIC AIRWAY NEURONS IN FERRET TRACHEA Z-X Wu[1], B.E. Satterfield[1], J.S. Fedan[2], R.D. Dey[1]. Department of Neurobiology and Anatomy, West Virginia University[1], Morgantown, WV 26506 and PPRB, NIOSH[2], Morgantown, WV 26505.

Exposure to ozone (O_3), a major air pollutant in urban areas, induces airway hyperresponsiveness mediated partly by the release of tachykinins from nerve terminals of intrinsic airway ganglia (Wu et al., J. Appl. Physiol. 91:371-378, 2001). The purpose of this study was to investigate the possible involvement of intrinsic airway neurons in O_3-induced airway hyperresponsiveness. Segments of ferret trachea were exposed *in vitro* to 2 ppm O_3 or air for 1 h. Reactivity of isolated tracheal smooth muscle strips to methacholine was significantly increased after O_3-exposure, as were contractions to electrical field stimulation (EFS). The O_3-enhanced responsiveness was maintained in tracheal segments cultured for 24 h, a procedure shown to deplete most sensory nerves while maintaining viability of intrinsic airway neurons. Furthermore, segments of trachea cultured with 3×10^{-6} M capsaicin for 24 h, which completely depletes tachykinins in sensory nerves, did not abolish the O_3-enhanced reactivity to cholinergic agonists and EFS. The findings support the hypothesis that O_3-induced airway smooth muscle hyperresponsiveness results partly from the activation intrinsic airway neurons. Further, these neurons may be activated by reflexes or mediators originating in the airway wall.

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Activation of Lung Vagal Sensory Receptors by Aerosolized H_2O_2 in Rats. Ting Ruan and Yu Ru Kou. Institute of Physiology, School of Medicine, National Yang-Ming University, Taipei, 11211 Taiwan.

Reactive oxygen species have been implicated in the pathogenesis of various pulmonary diseases. We recently reported that inhalation of aerosolized H_2O_2 evokes airway reflexes that are mediated through lung vagal afferents. This study investigated the effects of aerosolized H_2O_2 on the afferent activity arising from vagal pulmonary C fibers (CFs), rapidly adapting receptors (RARs), and pulmonary stretch receptors (PSRs) in 31 anesthetized, paralyzed, and artificially ventilated rats. Delivery of aerosolized H_2O_2 (0.5%, 25 tidal breaths) stimulated 9 of the 13 CFs, 7 of the 8 RARs, and 1 of the 10 PSRs studied. The stimulation of CFs (baseline vs. peak response: 0.18±0.08 vs. 12.02±1.31 imp/s, n=13) started 5-15 s and reached its maximum at 8-37 s after H_2O_2 challenge. The stimulation of RARs (baseline vs. peak response: 1.41±0.43 vs. 9.71±1.72 imp/breath) started 9-40 s and reached its maximum at 20-92 s after H_2O_2 challenge. The evoked discharge of CFs was not in phase with the ventilatory cycle, whereas that of RARs showed a respiratory modulation. A repeated challenge of H_2O_2 produced similar afferent responses in the same pulmonary receptors. Our results suggest that both CFs and RARs may function as an important afferent system during pulmonary insult by H_2O_2.

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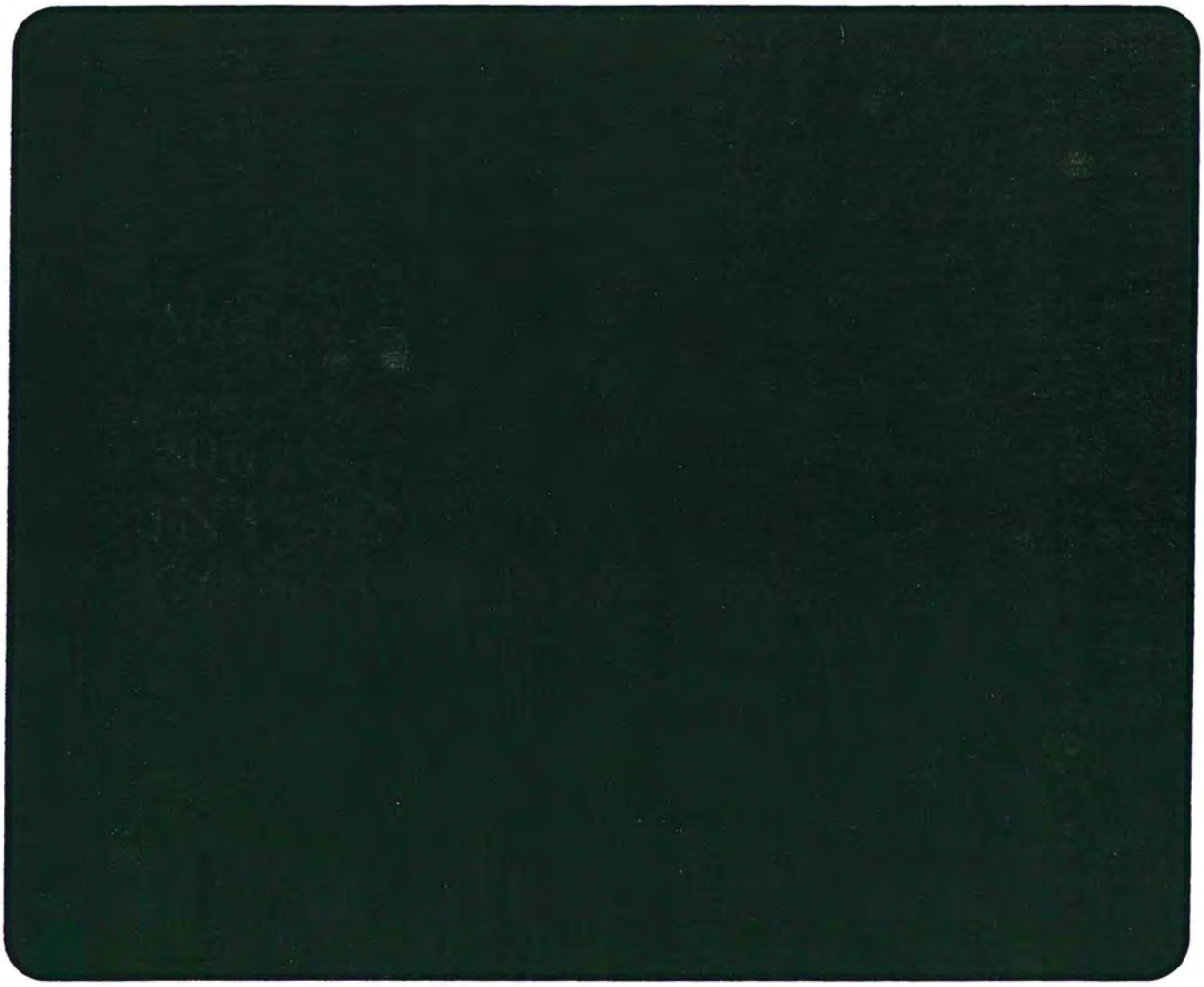

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