C1-61

Anthocyanins inhibit airway inflammation and hyperresponsiveness in a murine asthma model

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Asthma is a common chronic inflammatory disease regulated by coordination of T-helper cell type 2 (Th2) cytokines and inflammatory signal molecules. Additionally, oxidative stress may play an important role in airway inflammation such as eosinophilia, mucus hypersecretion, and airway hyperresponsiveness (AHR). In the present report, we investigated whether anthocyanins would reduce airway inflammation in a mouse asthma model immunized and challenged with ovalbumin (OVA). OVA inhalation elicited inflammatory responses characterized by eosinophilia and increased lipid hydroperoxide (LPO) in bronchoalveolar lavage (BAL) fluid, enhanced pause (Penh), increased glycoprotein and proliferating cell nuclear antigen (PCNA) expressions in mucus hypersecretion, and an increased expression of various cytokines and cyclooxygenase (COX) 2 in lung tissues. All parameters were attenuated in a dose-dependent manner by the administration of anthocyanins. These results suggest that anthocyanins may attenuate the development of asthma by downregulating Th2 cytokines, proinflammatory cytokines, and COX-2. Our findings suggest that anthocyanins have positive contributions as a dietary supplement for the prevention of asthma.

C1-62

Phospholipase D is important in Der f 2 induced expression of IL-8 and IL-13 in human bronchial epithelial cells

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The purpose of this study was to identify the role of PLD in Der f 2 induced IL-8 and IL-13 expression. The major house dust mite allergen, Der f 2, stimulates the PLD in human bronchial epithelial cell line (BEAS-2B). PLD activity was increased within 5 min after exposure of Der f 2. The well-known PLD activator PKC-z was found to be translocated to membrane from cytosol in Der f 2 treated BEAS-2B cells. To determine whether the effects of Der f 2 on PLD occurred as a consequence of PKC activation, BEAS-2B cells were pretreated for 30 min with PKC inhibitor (RO320432). RO320432 reduced the effects of Der f 2 induced PLD activation suggesting that PKC-z acts as upstream activator of PLD in Der f 2 treated BEAS-2B cells. Also, the p38 MAPK inhibitor (SB203580) prevented PLD activation. Der f 2 enhanced IL-8 and IL-13 expressions in BEAS-2B cells. We found that the expressions of IL-8 and IL-13 were increased when PLDs were activated with Der f 2 in BEAS-2B cells. To confirm the role of PLD in IL-8 and IL-13 expression, we transfected the PLD1 and PLD2, and their dominant negative forms. Interestingly, we found that only PLD1, not PLD2, overexpressed IL-8 and IL-13. These results indicate that Der f 2 might activate PLD through PKC-z activation and p38 MAPK phosphorylation which induces IL-8 and IL-13 expression in BEAS-2B cells.

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Synergistic effects and reversible inhibition of cAMP-dependent protein kinase catalytic subunit

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Asymmetric and synergistic interactions between cAMP-dependent protein kinase catalytic subunit, its substrates (ATP and kemptide) and inhibitors (H-89, kemptide Ala-analogue LRRAALG-NH2, and peptide-nucleoside conjugate inhibitor Adc/AhxArg6) were quantified in terms of binding effectiveness of these ligands with the free enzyme, the enzyme-ATP and enzyme-kemptide complexes. A simple kinetic procedure was proposed for characterization of these interactions, by using the second-order rate constants, calculated from the steady-state reaction kinetics. This procedure avoids complications related to the complex catalytic mechanism of the protein kinase catalyzed reaction. It was found that in some cases synergistic enhancement of ligand binding occurs in the presence of substrates. This phenomenon is typical for synergistic interaction between ligands and the enzyme. The principle “better binding - stronger synergism” was formulated for cAMP-dependent protein kinase catalytic subunit on the basis of this analysis and some linear-free-energy relationships between synergistic effect and ligand affinity were discovered.

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Ghrelin signaling to ERK 1/2: role of G-proteins and beta-arrestins

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Ghrelin, an acylated peptidyl gastric hormone, regulates GH release, food intake and energy homeostasis and exerts other functions including effects on cell proliferation through the activation of the MAPK cascade. The signaling pathways associated to the activation of MAPK were investigated in HEK 293 cells stably transfected with the ghrelin receptor GHS-R1a. One pathway is mediated by the βarrestins 1 and 2, and requires entry of the receptor into a multiprotein complex with the βarrestins, Src, Raf-1, and ERK 1/2. A second pathway is Gαq-dependent and involves a PKCα/β and Src. A third pathway is Gβγ-dependent and involves PKD, PKCζ, and Src. Our study reveals that Gαs and Gα15Proteins are crucially involved in the β-arrestin-mediated ERK 1/2 activation. Acknowledgements: This work was supported by grants from the FIS and the Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo and the Secretaria Xeral de Investigacion e Desenvolvemento, Xunta de Galicia (Spain).