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Cross-regulation between IL-3 and PKC-δ in lymphocytes: the effect on actin cytoskeleton.

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Regulation of actin cytoskeleton in lymphoid cells is poorly known. We show that in murine pre-B lymphoid cell line Baf3, the presence of IL-3 is required for the formation of membrane ruffles that intensely stain for actin and are responsible for the elongated cell phenotype. Withdrawal of IL-3 dissolves ruffled protrusions and converts the cell phenotype to round. Flow cytometric analysis of the cell shape showed that an inactive analog of Rac1, but not inactive RhoA or inactive cdc42 rounds the cells in the presence of IL-3. Constitutively activated Rac1 restores the elongated cell phenotype to in IL-3-starved cells. We conclude that the activity of Rac1 is necessary and sufficient for the IL-3-induced assembly of membrane ruffles. Similar to the IL-3 withdrawal, phorbol 12-myristate 13-acetate (PMA) dissolves actin-formed membrane ruffles and rounds the cells in the presence of IL-3. Flow cytometric analysis of the cell shape demonstrated that in the presence of IL-3 the PMA-induced cell rounding can not be abolished by constitutively active Rac1, but can be imitated by inactive Rac1. These data indicate that PMA disrupts the IL-3 pathway downstream of Rac1. Cells rounded by PMA return to the elongated phenotype concomitantly with PKC depletion. PMA-induced cell rounding can be reversed by the PKC-specific inhibitor GF109203X. Experiments with overexpression in Baf3 of individual PKC isoforms and a dominant-negative PKC-δ indicate that activation of PKC-δ, but not other PKC isoforms is responsible for disruption of membrane ruffles.

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Functional specificity deriving from a unique plasticity of fully α-helical Ras and Rho gaps

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GTPase-activating proteins (GAP) play a crucial role in regulating Ras/Rho small GTPases mediated signalling pathways. In the present study, sequence and structure comparisons of p120Ras and p50Rho GAPs evidenced that the α-helix packings constituting the two GAP interacting domains are related and mainly composed of two modules: a flexible four-helix bundle and a three-helix core we termed "cradle fold". The structural comparisons of the two binary complexes (i.e. p120/Ras and p50/Rho) allowed the identification of specific structural features such as helix A1 of p50 and α8c of p120, assumed to be involved in respective small GTPase recognition. We have identified important residues, that may be critical for the stabilization of the GAP/GTPase complexes, beside the conserved "arginine fingers". In addition, the flexible four-helix bundle common to the two GAPs is characterized by the identification of conserved topohydrophobic positions. Altogether, these data are consistent with a rearrangement of several helices around a common core: this represents a feature shared by p50 and p120 which thus might derive from a unique fold possessing a remarkable flexibility. Finally, the proposed overal plasticity helps understand both the function similarity and the function specificity displayed by the two modulator proteins.

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Role of Cyclic AMP in Stimulation of Cdc42 by A_{2A} and A_{2B} Adenosine Receptors in Human Mast Cells.

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Cdc42 is proposed to play a role in mast cell secretion. We examined its role in adenosine-induced mast cell activation. Human HMC-1 mast cells express both A_{2A} and A_{2B} subtypes of adenosine receptors, both known to increase intracellular cAMP levels. We found that A2 adenosine receptors activate Cdc42 in HMC-1 cells. The maximal activation of Cdc42 was reached within one minute incubation with the non-selective A_{2A}/A_{2B} agonist NECA. The selective A_{2A} agonist CGS21680 (1 μM) also activated Cdc42 and this effect was blocked by the selective A_{2A} antagonist ZM241385. However, activation of Cdc42 via A_{2B} receptors with 10 μM NECA was not blocked by ZM241385, indicating that both A_2 subtypes of adenosine receptors can activate Cdc42. Furthermore, stimulation of adenylate cyclase with forskolin, or loading of HMC-1 with the cell-permeable cAMP analog 8-Br-cAMP also activated Cdc42. The selective protein kinase A inhibitor H-89 reversed the efffects of NECA and CGS21680 on Cdc42, indicating the involvement of PKA in activation of Cdc42 by adenosine A2 receptors. Finally, we showed that a dominant negative mutant of Cdc42 attenuated NECA-induced IL-8 gene expression, indicating a possible role for Cdc42 in adenosine signaling.(Support: NIH R29HL55596).

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GPCR activation of Rho in 1321N1 cells: Relationship to change in cell morphology

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The small G protein Rho is a mediator of GPCR-stimulated cytoskeletal rearrangement, contractility, gene expression and cell growth. We previously reported that thrombin, but not carbachol, induced Rho-dependent DNA synthesis, cell rounding and process retraction in 1321N1 astrocytoma cells. In the present study activation of Rho was directly assessed to explore differential regulation by thrombin and carbachol. GTP-bound Rho was detected by its specific binding to a GST-fusion protein of the Rho binding domain (RBD) of rhotekin. In intact 1321N1 cells thrombin, but not carbachol, increased Rho-RBD binding 5-30 fold, consistent with the selective effect of thrombin on Rho-mediated responses. We also evaluated the ability of the GPCR agonist lysophosphatidic acid (LPA) to elicit DNA synthesis, cell rounding and Rho activation in 1321N1 cells. Interestingly while LPA and thrombin induced equivalent increases in Rho activation and DNA synthesis, LPA did not elicit cell rounding. Since we have shown that microinjection of activated Rho is sufficient to induce cell rounding, the failure of LPA to do so suggests that this agonist may signal through an additional parallel pathway to inhibit Rho-mediated changes in morphology. We are currently examining the effects of elevating cAMP on Rho activation, since pretreatment with forskolin/IBMX inhibits thrombin-stimulated changes in cell morphology. Preliminary results suggest that forskolin/IBMX inhibits basal and thrombin-mediated activation of Rho. Further studies are aimed at revealing the mechanisms of and G proteins involved in Rho activation in response to receptor stimulation.

SMOOTH MUSCLE PHARMACOLOGY/TOXICOLOGY (1068-1069)

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AGE-DEPENDENT SWITCH IN EXPRESSION OF HUMAN Ca^{2+} CHANNEL α_{1C} SUBUNIT ISOFORMS. Z. Zhang, D. R. Abernethy and N. M. Soldatov. Natl. Inst. on Aging, NIH, Baltimore, MD 21224.

Expression of splice variants of the L-type Ca^{2+} channel pore-forming α_{1C} subunit is controlled by yet poorly investigated biochemical stimuli which may underlie physiological and metabolical abnormalities associated with diseases. Exon 21 of the α_{1C} gene contributes to weaker inhibition of Ca^{2+} channel by isradipine at negative potentials (Soldatov et al., *J. Biol. Chem.* **270:** 10540, 1995). In this study, we investigated whether exposure of normal human aortic smooth muscle cells from donors ranging from 0.3 to 61 years of age to a variety of stimuli affects the expression of the α_{1C} splice variants. Cells were grown in a standard medium with 10% fetal calf serum to ≈ 30% confluence and were then synchronized by serum-deprivation for 48 h. Poly(A+) mRNA was isolated and the expression of alternative exons was analyzed by RT-PCR. We found that exon 22-isoform of α_{1C} is predominantly expressed in these cells. Serum deprivation induced a notable expression of exon 21-isoform of α_{1C} only in cells from older (54, 57, 59 and 61 years old) donors. An opposite effect of endothelin and to less extent of angiotensin on expression of 21-isoform of α_{1C} was found in some cells. Splicing patterns of alternative exons 31/32/33 and 8/8A were not significantly affected by the tested stimuli. Our data suggest that expression of the exon 21-isoform of α_{1C} occurs in response to reduction of proliferative signals in an age-dependent manner and is in part responsive to hormonal stimuli known to control intracellular signaling in vascular smooth muscle cells.

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LIPOPOLYSACCHARIDE-INDUCED AIRWAY HYPOREACTIVITY TO METHACHOLINE IN GUINEA PIGS *IN VIVO* IS ABSENT IN THE ISOLATED, PERFUSED TRACHEA *IN VITRO*. *†R.A. Johnston, †S.G. Olson, and *†J.S. Fedan. *Dept. of Pharmacology and Toxicology, West Virginia University, Morgantown, WV 26506, and †Pathol. and Physiol. Res. Branch, HELD, NIOSH, Morgantown, WV 26505.

This study examined the effects of lipopolysaccharide (LPS)-treatment (4 mg/kg, i.p.; 18 hr post-exposure) on guinea pig reactivity to methacholine (MCh) *in vivo* and *in vitro*. *In vivo* airway reactivity to MCh was measured by placing conscious, guinea pigs in a two-chamber whole body plethysmograph for measuring specific airway resistance (SRaw) and exposing them to increasing concentrations of MCh aerosol. In LPS-treated animals, the basal SRaw was increased compared to that in the saline-injected control animals. In addition, the LPS-treated animals were hyporeactive to inhaled MCh. Airway reactivity *in vitro* was measured using the isolated, perfused trachea preparation to permit MCh addition separately to the serosal (extraluminal; EL) or mucosal (intraluminal; IL) surfaces; because of the epithelium, EL reactivity to MCh is greater than IL reactivity. In epithelium-intact tracheas there was no difference in the EC_{50} values for contraction to EL and IL MCh between control- and LPS-treated guinea pigs. In epithelium-denuded tracheas where the difference between EL and IL reactivity was abolished, LPS-treatment had no effect on reactivity to MCh. These results suggest that the hyporeactivity observed *in vivo* does not involve the trachea or the mechanism(s) is lost *in vitro*.

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ABSTRACTS

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