[PA1-46] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Comparative studies of molecular mechanisms of dopamine D2R and D3R receptors for the activation of mitogen activated protein kinase in HEK-293 cells

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Dopamine $D_2$and D_3 receptors (D_2R and D_3R) belong to G protein coupled receptor family. They are co-localized in some regions of brain, and share similar structural and functional characteristics, for example, activation of ERK1/2. In this study, we conducted series of experiments to understand the differential regulatory processes underlying D_2R/D_3R-mediated activation of ERK1/2. For both D_2R/D_3R-mediated ERK1/2 activation, pertussis toxin-sensitive G proteins were involved, and treatments that inhibit clathrin-mediated receptor endocytosis (sucrose, dominant negative mutants, dynamin-K44A or β-arrestin1-V53D) did not affect them. In additon, wortmannin, a specific inhibitor of phosphatidylinositol 3-kinase and Go6983, a PKC isotype-specifc inhibitor also abolished both D_2R/D_3R mediated ERK1/2 activation. Interestingly, tryphostin AG1478, a selective inhibitor of tyrosine kinase of epidermal growth factor receptor and ΔN-Raf (dominant negative mutant of $p74_{raf-1}$) effectively blocked D_2R-mediated ERK1/2 activetion, but not that mediated by D_3R. These results suggest that D_2R activates ERK1/2 through classical MAPK cascades involving transactivation with EGFR, however, D_3R uses distinct signaling pathways for the ERK1/2 activation.

[PA1-47] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

The involvement of benzodiazepine receptor on the relaxation of cat lower esophageal sphincter tone

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It has been previously suggested that NO and vasoactive intestinal polypeptide (VIP) are neurotransmitters which control Lower esophageal sphincter (LES) and esophageal peristalsis. However, it is also proposed that another transmitter(s) is involved in LES relaxation.
In the present study, we investigated the effect of GABA and benzodiazepine on the VIP- or electrical field stimulation (EFS)-induced relaxation in cat LES muscle.
GABA, muscimol (GABAa agonist), and baclofen (GABAb agonist) had no effects on the relaxation, which is induced by VIP or EFS. Bicuculline (GABAa antagonist) and phaclofen (GABAb antagonist) also had no effect on the relaxation, which is induced by VIP or EFS. However flumazenil (benzodiazepine antagonist) inhibited the VIP-induced LES relaxation, but had no effect on the EFS-induced LES relaxation.
Our results suggest that benzodiazepine receptor participates in the LES relaxation, which is mediated postsynaptically, but not presynaptically, via the interaction with VIP.

[PA1-48] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

The alteration of intracellular signaling on the smooth muscle cells relaxation in cat esophagitis

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We investigated the alteration of signal transduction on VIP-induced relaxation in cat esophagitis. Acute esophagitis (AE) was induced by perfusion with 0.1N HCl at a rate of 1 ml/min for 45 min over three consecutive days. We have isolated smooth muscle cells of esophagus by enzymatic digestion with collagenase F.
After pretreatment of ACh, we compared relaxation of normal cells with those of esophagitis. VIP produced dose-dependent relaxation in normal cells, and this relaxation curve was down shifted when compared with those of esophagitis cells. SNP or SIN-1, which is a NO donor, produced the dose-dependent relaxation in normal cells, but there is no difference as compared with esophagitis. Forskolin (cAMP activator) or db-cAMP (cAMP analog) produced dose-dependent relaxation in normal cells, and this relaxation curve was down shifted when compared with those of esophagitis cells. The relaxation of esophagitis cells is reduced by 20% as compare with normal cells. 8-Br-cGMP (cGMP analog) induced dose-dependent relaxation, but there is no difference between normal and esophagitis.
This result suggests that cAMP dependent pathway rather than cGMP dependent pathway plays a role on the regulation of VIP induced relaxation in cat acute esophagitis.

[PA1-49] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Novel ginseng saponine metabolite induces apoptosis through activation of caspase-8, BID cleavage and cytochrome c release in HepG2 cells

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The novel intestinal bacterial metabolites of ginseng protopanaxadiol saponins 20-O-(ß-D_glucopyranosyl)-20(S)-protopanaxadiol (IH-901) formed from ginsenosides Rb1, Rb2 and Rc, is reported to be a potential chemopreventive and chemotherapeutic agent. We show here that IH-901 induced apoptosis in human hepatoblastoma HepG2 cells as determined by morphological analysis, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining, DNA fragmentation and flow cytometric analysis. The apoptosis by IH-901 induced through mitochondrial pathway involving caspase-8, Bid cleavage, cytochrome c (cyt c) release and caspase-3 activation. Caspase activation was a necessary requirement for apoptosis by IH-901 because the pretreatment with the broad-spectrum caspase inhibitor (zVAD-fmk, 50 uM) and specific caspase-8 inhibitor (zIETD-fmk, 10um) for 18h increased cell viability to 55 % and 47 %, 1.7- or 1.5-fold compared with the IH-901 only ($p < 0.01$, Student t-test). The decrease in the cell death by pretreatment with antagonistic anti-Fas antibody (ZB4) and the activation of the initiator caspase-8 indicated that IH-901 induced signaling pathway requires the Fas death receptor. Though IH-901 did not induce Fas or FasL mRNA and protein expression, it appeared that the cleavage of cytosolic BID by caspase-8 to truncated tBID. tBID translocated to the mitochondria to induce the oligomerization results in the cytc release in a time-dependent manner, whereas antiapoptotic mitochondrial Bcl-x decreased in a time-dependent manner. Primary hepatocytes isolated from normal Sprague-Dawley rats are not affected by IH-901 (60uM). The very low toxicity in normal hepatocytes and its high activity in hepatoblastoma HepG2 cells suggest that IH-901 is a promising experimental cytotoxic agent.
Our results indicated that IH-901 induces apoptosis through caspase-8, BID cleavage, cyt c release, caspase-3 and PARP activation. These results also suggest that oligomerization of tBID plays a critical regulator the release of cytc.

[PA1-50] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Ginsenoside-Rh1 and Ginsenoside-Rb1 display estrogenic activity in human breast carcinoma MCF-7 cells.

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