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α_2-Adrenergic Receptors Activate MAPK *via* PI-3K Activation and EGF Receptor Transactivation in PC12 Cells

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S u m m a r y. We have previously shown that stimulation of all three α_2-AR subtypes in PC12 cells leads to p42/p44 MAPK (ERK 1/2) activation (1). As an initial effort to delineate the signal transduction mechanisms employed by the α_2-ARs for this effect, we investigated the involvement of PI-3K and EGF receptor and found that the activation of MAP kinase by α_2-ARs in PC12 cells requires transactivation of the EGF receptor and activation of PI-3K. These results reflect the remarkable diversity of the signal transduction pathways utilized by α_2-ARs in order to elicit the critical signaling event of MAPK activation in PC12 cells.

INTRODUCTION

The α_2-adrenergic receptors (α_2-ARs) belong to the superfamily of G-protein-coupled receptors (GPCRs). They mediate effects of the endogenous catecholamines epinephrine and nor-epinephrine. Three different genes encode the human α_2-adrenergic subtypes (α_{2A}, α_{2B} and α_{2C}), which differ in their ligand binding properties, tissue distribution, chromosomal localization and signaling pathways (2).

Recent studies have demonstrated that growth factor receptors and GPCRs may both activate the same effector molecules and utilize the same signaling cascades in cells. One of the most common signaling events mediated by both types of receptors is the activation of mitogen-activated protein (MAP) kinase, although portions of the signaling cascade between receptor and MAP kinase can be different for growth factor receptors and GPCRs.

We have previously shown (1) that stimulation of all three α_2-AR subtypes in PC12 cells leads to p42/p44 MAPK (ERK 1/2) activation. The signaling mechanisms by which α_2-ARs activate the MAPKs are only poorly characterized. Gi/Go coupled receptors, such as the α_2-ARs, have been shown to activate MAPK through the $G\beta\gamma$ subunits released from activated, pertussis toxin-sensitive Gi/Go proteins, in a process that does not require receptor internalization (3). PI-3K, a kinase that is critically important for cell survival, has been shown to participate in α_2-AR- and in other Gi/Go coupled receptor-induced MAPK activation, as well (4).

GPCRs have also been shown to transactivate growth factor receptors, including the EGF receptor and the PDGF receptor (5) in several cell lines. Several reports suggest that the EGF receptor and other proteins may serve as a scaffolding structure or as an adaptor protein to which other signaling proteins may be recruited in response to GPCR signaling (6).

In the present study we examined the activation of MAP kinase by α_2-ARs in stably transfected PC12 cells and particularly focused on the involvement of PI-3K and EGF receptor in this α_2-AR-induced effect. We found that the activation of MAP kinase by α_2-ARs in PC12 cells involves transactivation of the EGF receptor and activation of PI-3K.

MATERIALS AND METHODS

Cell Culture: PC12 cells stable expressing human α_2-ARs were grown on collagen-coated 35mm-diameter dishes at 37 ^{0}C in a 95% air, 5% CO_2 mixture, in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% HS and 5% FBS in the presence of antibiotics (50μg/ml streptomycin, 100IU/ml pencillin), 292 mg/l L-

glutamine, 1 mM sodium pyruvate and 20 mM $NaHCO_3$.

Western Blotting: For the MAPK analysis PC12 cells were cultured in DMEM containing 10% HS and 5% FBS, which was removed and replaced by serum-free medium for four hours before the experiment. Tyrphostin AG1478, LY294002 were added to the serum-free medium 40 min before the addition of the regulator substances epinephrine (10 μM), EGF (50 ng/ml) and NGF (100 ng/ml). Cells were lysed on ice in 100 μl of SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM DTT, 0,1% bromophenol blue) and the lysates were separated by 10% SDS-PAGE, followed by electroblotting. Dublicate nitrocellulose blots were blocked in Tris-buffered saline (TBS)/0.1 % Tween 20, containing 5 % non-fat dry milk, and incubated with phospho-specific MAPK, p44/42 MAPK (total) and then with horse radish peroxidase-conjugated goat anti-rabbit antibody. Immunostained proteins were visualized using the ECL detection system (Amersham Pharmacia Biotech).

RESULTS

α_2-AR-induced MAPK Phosphorylation requires EGF-R Transactivation.- In an effort to explore the signaling mechanism of α_2-AR-induced MAPK activation in PC12 cells and to test the potential involvement of EGF-R transactivation in this effect, we performed Western blotting on extracts from α_{2C}-AR expressing PC12 cells, treated with epinephrine alone or with epinephrine in the presence of the specific inhibitor of EGF-R tyrosine kinase activity, tyrphostin AG1478. As shown in Figure1, the pre-incubation of PC12 cells in culture medium containing 500 nM Tyrphostin AG1478 almost totally prevented the phosphorylation of MAPK caused by epinephrine. Thus, the transactivation of EGFR plays a critical role in the mediation of the effect of α_{2C}-AR on MAPK. Similar results were obtained in the other two α_2-AR subtype-expressing PC12 clones (data not shown).

α_2-AR-induced MAPK Phosphorylation is mediated by PI-3K.- To test the involvement of PI-3K in α_{2C}-AR-induced MAPK activation in PC12 cells, we performed similar, as above, experiments in α_{2C}-AR expressing PC12 cells, using a PI-3K specific inhibitor, LY294002. As shown in Figure 2, the epinephrine-induced MAPK activation is abolished in the presence of LY294002. Similar results were obtained in the other two α_2-AR subtype-expressing PC12 clones (data not shown). These results suggest that PI-3K mediates the signaling of all three α_2-AR subtypes to ERK 1/2 activation in PC12 cells.

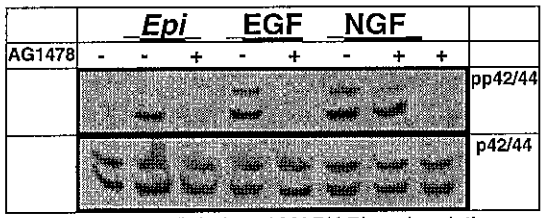

Figure 1. α_2-AR-induced MAPK Phosphorylation requires EGF-R Transactivation

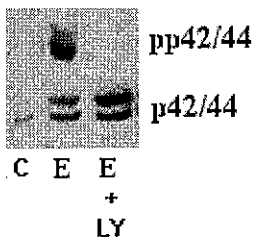

Figure 2. α_{2C}-AR-induced MAPK phosphorylation is mediated by PI-3K in PC12 cells

CONCLUSIONS

We had previously shown that all α_2-ARs activate MAPK in PC12 cells (1). The present study shows that this activation involves transactivation of the EGF receptor and activation of PI-3K. These results suggest that the α_2-ARs, like many other GPCRs, utilize receptor tyrosine kinases for their signaling in PC12 cells, as well. PI-3K has been suggested to be an early intermediate in GPCR signaling to MAPK in various cell lines. Our results show that this might be the case in PC12 cells, as well. One very interesting question, raised by the results of this study and requiring further investigation, is whether PI-3K is upstream, downstream, or independent of EGFR transactivation in the signaling of α_2-ARs to MAPK in PC12 cells. This is the focus of our current investigations.

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