

Direct Linkage of Three Tachykinin Receptors to Stimulation of Both Phosphatidylinositol Hydrolysis and Cyclic AMP Cascades in Transfected Chinese Hamster Ovary Cells*

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The mammalian tachykinin system consists of three distinct peptides, substance P, substance K, and neuropeptide K, and possesses three corresponding receptors. In this investigation we examined intracellular signal transduction of the individual tachykinin receptors by transfection and stable expression of these receptor cDNAs in Chinese hamster ovary cells. The three receptors commonly showed a rapid and marked stimulation in both phosphatidylinositol (PI) hydrolysis and cyclic AMP formation in response to tachykinin interaction. Direct linkage of the three receptors to both phospholipase C and adenylate cyclase was evidenced by the finding that tachykinins, added together with GTP, activated these enzyme activities in membrane preparations derived from tachykinin receptor-expressing cells. The stimulation of cyclic AMP formation was less efficient than that of PI hydrolysis in receptor-expressing cells as well as their membrane preparations (about 1 order of magnitude difference in the effective peptide concentrations). However, the stimulatory responses of the PI hydrolysis and cyclic AMP formation in both receptor-expressing cells and their membrane preparations were induced in complete agreement with the tachykinin binding selectivity of each subtype of the receptors. This investigation demonstrated unequivocally that the tachykinin receptors have the potential to couple directly to both phospholipase C and adenylate cyclase and to stimulate PI hydrolysis and cyclic AMP formation.

The mammalian tachykinin system represents a typical example of biologically active peptides which exhibit a high degree of functional diversity at the level of both peptide production and peptide reception (1, 2). This system consists of three distinct peptides, substance P, substance K (neurokinin A) and neuropeptide K (neurokinin B), and possesses three different types of receptors, each specific for the respective peptide (1, 2). In our previous studies we reported the molecular cloning of functional cDNAs for the substance P,

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substance K, and neuromedin K receptors (SPR, SKR, and NKR) and the characterization of the cloned receptors expressed in *Xenopus* oocytes and in mammalian cells (3-6). The molecular cloning and characterization of the rat substance P receptor have also been reported by Hershey and Krause (7). The three tachykinin receptors belong to the family of guanyl nucleotide-binding regulatory protein (G protein)-coupled receptors but differ in affinities for the three tachykinins and in expression sites of their mRNAs (4-8). The different physiological responses of the three tachykinins thus occur as a result of the selectivity and different distribution of the three receptors.

The three tachykinin receptors have been shown to mediate the stimulation of phosphatidylinositol (PI) hydrolysis in many tissue preparations and different cell types (9-11). Recently the cDNA for bovine substance K receptor was transfected into murine fibroblasts, and it was shown that this receptor indeed stimulates PI hydrolysis after the application of tachykinin peptides to these cells (12). Intracellular signal transduction involved in the tachykinin receptors, however, seems to be more complex because it has been reported that tachykinin peptides stimulate cyclic AMP generation in some tissues but not in others (10, 13, 14) and even inhibit cyclic AMP formation (15). Furthermore, the precise characterization of signal transduction underlying individual tachykinin receptors has been limited in tissue or cell preparations because of the presence of multiple tachykinin receptors (8) and the lack of specific agonists and antagonists. The transfection and functional expression of the cDNA clone for each tachykinin receptor into the same cell type provide a model system with which to study precisely signal transduction of a single receptor subtype without any ambiguity because of the presence of multiple receptor subtypes of the same receptor family. In this investigation we stably expressed individual rat tachykinin receptors by DNA transfection into Chinese hamster ovary (CHO) cells and investigated detailed intracellular transduction mechanisms of each of the three tachykinin receptors.

EXPERIMENTAL PROCEDURES

Materials.—Materials were obtained from the following sources: a minimal essential medium and a minimal essential medium lacking ribonucleosides and deoxyribonucleosides were from GIBCO; fetal

The abbreviations used are: SPT, substance P receptor; SKR, substance K receptor; NKR, neuropeptide K receptor; PI, phosphatidylinositol; IP₃, inositol triphosphate; IP₄, inositol tetraphosphate; IP₅, inositol triphosphate; PBS, phosphate-buffered saline; CHOD, Chinese hamster ovary; IBMX, 3-isobutyl-1-methylxanthine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; G protein, guanyl nucleotide-binding regulatory protein; EGTA, ethylene bis(oxyethyletriamine)tetraacetic acid.

Tachykinin Receptors

J Elliott

Tachykinin Receptors:

The Tachykinin Receptors Stephen H. Buck, 2012-12-06 Offers a state of the art review by international experts on all aspects of tachykinin receptors including neuropeptide peptide and G protein linked receptors in general. It covers the physiology pharmacology biochemistry and molecular biology of these receptors from both clinical and basic research points of view. Topics treated by the distinguished contributors include the characterization of tachykinin receptors the mechanisms of tachykinin receptor action a reflection on future prospects and a historical consideration of tachykinin research.

Tachykinins Peter Holzer, 2012-12-06 The tachykinins represent one of the most thoroughly investigated family of neuropeptides whose members and receptors have been characterized at the genetic and molecular level and whose pharmacology has now been advanced to the first clinical application. These exciting accomplishments and prospects are reviewed and discussed in this volume in an authoritative manner. Particular emphasis is laid on the development of selective non peptide antagonists for all 3 tachykinin receptors and their potential as novel drugs in a variety of diseases. The approval of the first tachykinin receptor antagonist as an antiemetic drug is particularly highlighted and the utility of tachykinin receptor antagonists in affective disorders chronic obstructive airway disease and irritable bowel syndrome to name a few indications is extensively considered.

Receptors P. Michael Conn, 1993 Receptors initiate the means by which cellular regulators exert their actions on targets. Because of the central role of cell cell communication and signal transduction receptors are of intrinsic interest to neuroscientists. Receptor studies utilize both traditional methods of analysis and modern molecular techniques. Key Features: Methods presented for easy adaptation to new systems. Comprehensive protocols included for molecular techniques PCR cloning transfection coupling techniques for the determination of receptor subclasses. Techniques for localization in situ hybridization immunocytochemistry ligand design radioactive techniques biotinylated techniques receptor associated kinase. Methodology described for the following receptors: acetylcholine angiotensin II, bombesin, GRP, dopamine, GABA, G protein coupled receptors, neurotensin, NGF, NPY, serotonin, somatostatin, tachykinin.

Cumulated Index Medicus, 1995 **Characterization of Multiple Modes of Tachykinin Receptor Adaptive Regulation** Chris Alexander Haskell, 1996 **New Perspectives in Monitoring Lung Inflammation** Paolo Montuschi, 2004-10-14 Attracting the attention of the medical community exhaled breath condensate is a completely non invasive method for sampling secretions from the airways. Analysis of exhaled breath condensate is potentially useful for monitoring airway inflammation and in pharmacological therapy. With its non invasive nature this method may be suitable for longit.

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Verhandlungen der Deutschen Zoologischen Gesellschaft Deutsche Zoologische Gesellschaft,1994 List of members in each vol **The G-Protein Linked Receptor Facts Book** Steve P. Watson,Steve Arkinstall,1994-02-28 Catalogues major facts about receptors G proteins and effector molecules Each entry has a common format using a minimum amount of text and contains information on the sequence gene structure distribution agonists antagonists and physiochemical properties of these proteins **Canadian Journal of Physiology and Pharmacology** ,2002-05 *Annals of the New York Academy of Sciences* Noel R. Rose,2011 *Polish Journal of Chemistry* ,2000

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