

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

(Received for publication, April 26, 1991)

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The mammalian tachykinin system consists of three distinct peptides, substance P, substance K, and neuromedin 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 tachykinin, 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 neuromedin 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,

substance K, and neuromedin K receptors (SPR,² 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 precise 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: α -minimal essential medium and α -minimal essential medium lacking ribonucleosides and deoxyribonucleosides were from GIBCO; fetal

* This work was supported in part by research grants from the Ministry of Education, Science, and Culture of Japan; the Ministry of Health and Welfare of Japan; and the Yamamotochi Foundation for Research on Metabolic Disorders. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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² The abbreviations used are: SPR, substance P receptor; SKR, substance K receptor; NKR, neuromedin K receptor; PI, phosphatidylinositol; IP₃, inositol monophosphate; IP₂, inositol bisphosphate; IP₄, inositol trisphosphate; PBS, phosphate-buffered saline; CHO, Chinese hamster ovary; IBMX, 3-isobutyl-1-methylxanthine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; G protein, guanyl nucleotide-binding regulatory protein; EGTA, [ethylenediamine]oxyethylene[*N*,*N*'-bis(triacetic acid)].

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Table of Contents Tachykinin Receptors

1. Understanding the eBook Tachykinin Receptors
 - The Rise of Digital Reading Tachykinin Receptors
 - Advantages of eBooks Over Traditional Books
2. Identifying Tachykinin Receptors
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Tachykinin Receptors
 - User-Friendly Interface
4. Exploring eBook Recommendations from Tachykinin Receptors
 - Personalized Recommendations
 - Tachykinin Receptors User Reviews and Ratings

- Tachykinin Receptors and Bestseller Lists
- 5. Accessing Tachykinin Receptors Free and Paid eBooks
 - Tachykinin Receptors Public Domain eBooks
 - Tachykinin Receptors eBook Subscription Services
 - Tachykinin Receptors Budget-Friendly Options
- 6. Navigating Tachykinin Receptors eBook Formats
 - ePub, PDF, MOBI, and More
 - Tachykinin Receptors Compatibility with Devices
 - Tachykinin Receptors Enhanced eBook Features
- 7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Tachykinin Receptors
 - Highlighting and Note-Taking Tachykinin Receptors
 - Interactive Elements Tachykinin Receptors
- 8. Staying Engaged with Tachykinin Receptors
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers Tachykinin Receptors
- 9. Balancing eBooks and Physical Books Tachykinin Receptors
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Tachykinin Receptors
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Tachykinin Receptors
 - Setting Reading Goals Tachykinin Receptors
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Tachykinin Receptors
 - Fact-Checking eBook Content of Tachykinin Receptors
 - Distinguishing Credible Sources

13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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