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Identification of adenylyl cyclase isoenzymes in CHO and B82 cells

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Abstract

The identification of adenylyl cyclase isoenzymes in mammalian host cells is important for the interpretation of data obtained from cell lines heterologously expressing G-protein coupled receptors. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to amplify adenylyl cyclase cDNAs from Chinese hamster ovary (CHO) and mouse fibroblast (B82) cells. The isolated fragments were identified by restriction analyses and by sequencing. We found mRNAs for adenylyl cyclases VI and VII in CHO and adenylyl cyclases IX and VII in B82 cells. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Chinese hamster ovary (CHO) cell; Mouse fibroblast (B82) cell; Adenylyl cyclase isoenzyme

Chinese hamster ovary (CHO) cells are the most frequently used mammalian host cell line for the expression of G-protein coupled receptor cDNAs. The murine fibroblast B82 cell line has also been used in our laboratory for the expression and characterization of the rat muscarinic acetylcholine receptor subtypes (Kashihara et al., 1992).

Mammalian cell lines have the correct cellular machinery for folding and posttranslational modification of the expressed receptor proteins. They are, therefore, useful tools for the screening of potential drug candidates in receptor binding assays. However, caution should be used in the interpretation of data concerning the signal transduction cascades mediated by the transfected receptors. Increasing number of studies show that the G-protein coupled receptors expressed in mammalian cell lines exhibit less specificity in their coupling properties than in their native environment (Kenakin, 1996). In addition, the outcome of each step in the signal transduction cascade is dependent on the regulatory properties of the particular isoenzyme pool present in the host cells. Ideally for the expression of a receptor protein a host mammalian cell line with an isoenzyme pool similar to that of the target tissue should be found. Consequently, the signal transduction cascade isoenzymes in both the target tissues and the host cells should be identified.

Nine isoforms of the mammalian adenylyl cyclases have been cloned. Several adenylyl cyclase families have been identified based on sequence similarities and regulatory properties (Sunahara et al., 1996). It has been suggested that the mammalian adenylyl cyclases work as 'molecular coincidence detectors' (Sunahara et al., 1996), so that their final reaction rate is determined by the integrated action of different intracellular modulators (pool of G-protein α subunits activated, concentration of free $\beta\gamma$ subunits, Ca^{2+} concentration, protein kinase A and protein kinase C activation, etc.). These intracellular modulators affect the adenylyl cyclase isoenzymes differently, therefore, the cAMP response upon the activation of a receptor is dependent on the cellular isoenzyme pool present in that particular cell.

In the present study, we have used the reverse transcriptase polymerase chain reaction (RT-PCR) method of Premont (1994) to identify adenylyl cyclase isoenzyme mRNAs expressed in CHO and B82 cells in order to properly interpret our cAMP formation data obtained from

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CHO cells transfected with opioid and B82 cells transfected with muscarinic and opioid receptors (Kashihara et al., 1992; Wei et al., 1994; Malatynska et al., 1996).

We isolated mRNA from CHO and B82 cells using the PolyATract mRNA isolation kit (Clontech, Palo Alto, CA). First strand cDNA was obtained using random primers and Superscript reverse transcriptase (Gibco/BRL, Gaithersburg, MD). Degenerate primers were synthesized (Integrated DNA Technologies, Coralville, TX) to the catalytic domain sequences KIKTIG (sense) and WGNTVN (antisense). The sequence of the PCR primers was: 5′ > CGGCAGCTCGAGAA(A/G)AT(A/C/T)AA(A/G)AC (I)AT(A/C/T)GG > 3′ (sense) and 5′ > CCGGGACTCGAGAC(A/G)TT(I)AC(I)GT(I)TT(I)CCCCA (antisense). The underlined sequences introduce *Xho*I restriction sites at both ends of the PCR product for subcloning. The PCR reaction was performed using 10 ng of first strand cDNA as a template. The reaction mixture was denatured (5 min at 94°C). After addition of 2.5 U *Taq* DNA polymerase 35 amplification cycles were performed: 95°C, 1 min (denaturation), 55°C, 1 min (annealing) and 72°C, 1 min (extension). The PCR product (0.3 kb) was isolated from a 2% agarose gel, digested with *Xho*I and ligated into the dephosphorylated *Xho*I site of pBS SK(−) using the Rapid DNA Ligation Kit (Boehringer-Mannheim, Indianapolis, IN). The ligation mixture was transformed into DH5α competent cells.

Sixty randomly selected white clones for each cell lines were sorted by restriction analysis using the following strategy: first screening: *Kpn*I/*Hin*cII; second screening: *Ava*II; third screening *Nco*I/*Eco*RI or *Apa*I/*Eco*RI. Several clones for each restriction pattern were sequenced using the Sp6 and T7 promoter regions of the vector. The sequences were identified using the BLAST search program (GeneBank, NCBI, MD) and also aligned with the published adenylyl cyclase sequences using the DNAsis program.

Thirteen clones with adenylyl cyclase VI restriction pattern obtained from the CHO cells mRNA were verified as adenylyl cyclase VI by sequencing. The sequence of the PCR fragment is 93.5% homologous to that of the corresponding published mouse adenylyl cyclase VI fragment (Yoshimura and Cooper, 1992). The homology of the deduced amino acid sequence is 100%, showing that our clones are presumably species homologs of the cloned mouse adenylyl cyclase VI. Several other clones were identified as adenylyl cyclase VII by restriction analyses of which two clones were verified as adenylyl cyclase VII by sequencing. The B82 cells also contain adenylyl cyclase VII by restriction analyses of which two clones were verified as adenylyl cyclase VII by sequencing. The majority of the clones from the B82 cells were identified as adenylyl cyclase IX by restriction digestion of which six clones were verified as adenylyl cyclase IX by sequencing.

These data are in excellent agreement with reported observations in that: (a) the maximal stimulation of cAMP formation by forskolin is much lower in B82 than in CHO cells (Kashihara et al., 1992; Malatynska et al., 1996), because the major isoenzyme in B82 cells—adenylyl cyclase IX—is weakly stimulated by forskolin (Yan et al., 1997); (b) the stimulatory component of the biphasic effect of muscarinic receptor agonists on forskolin stimulated cAMP formation in B82 cells transfected with the muscarinic M4 receptor (Kashihara et al., 1992) is due to the presence of adenylyl cyclase VII, an isoenzyme that is stimulated by high concentrations of the $\beta\gamma$ subunits of the G-proteins (Sunahara et al., 1996); (c) the prominent adenylyl cyclase superactivation after chronic inhibitory receptor stimulation (Malatynska et al., 1996) is due to the presence of adenylyl cyclase VI in transfected CHO cells and the lack of adenylyl cyclase superactivation under similar conditions in transfected B82 cells (Wei et al., 1994) is due to the absence of adenylyl cyclase VI from B82 cells.

In summary, the major adenylyl cyclase mRNAs are adenylyl cyclases VI and VII in CHO, and adenylyl cyclases IX and VII in B82 cells. This is the first study to identify adenylyl cyclase isoenzyme pools in these cell lines and provides important information for the interpretation of data obtained from the heterologous expression of different G-protein coupled receptors in these cell lines.

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References

Kashihara, K., Varga, E.V., Waite, S.L., Roeske, W.R., Yamamura, H.I., 1992. Cloning of the rat m3, m4, and m5 muscarinic acetylcholine receptor genes by the polymerase chain reaction and the pharmacological characterization of the expressed gene. Life Sci. 51, 955–971.

Kenakin, T., 1996. The classification of seven transmembrane receptors in recombinant expression systems. Pharmacol. Rev. 48, 413–463.

Malatynska, E., Wang, Y., Knapp, R.J., Waite, S.L., Calderon, S.N., Rice, K., Hruby, V.J., Yamamura, H.I., Roeske, W.R., 1996. Human delta opioid receptor: functional studies on stably transfected CHO cells following acute and chronic treatment with the selective nonpeptidic agonist SNC-80. J. Pharmacol. Exp. Ther. 278, 1083–1089.

Premont, R.T., 1994. Identification of adenylyl cyclases by amplification using degenerate primers. Methods Enzymol. 238, 116–127.

Sunahara, R.K., Dessauer, C.W., Gilman, A.G., 1996. Complexity and diversity of mammalian adenylyl cyclases. Annu. Rev. Pharmacol. Toxicol. 36, 461–480.

Wei, H.B., Yamamura, H.I., Roeske, W.R., 1994. Down-regulation and desensitization of the muscarinic M1 and M2 receptors in transfected fibroblast B82 cells. Eur. J. Pharmacol. 268, 381–391.

Yan, S.Z., Huang, Z.H., Rao, V.D., Hurley, J.H., Tang, W.J., 1997. Three discrete regions of mammalian adenylyl cyclase form a site for $G_{s\alpha}$ activation. J. Biol. Chem. 272, 18849–18854.

Yoshimura, M., Cooper, D.M.F., 1992. Cloning and expression of a Ca^{2+}-inhibitable adenylyl cyclase from NCB-20 cells. Proc. Natl. Acad. Sci. USA 89, 6716–6720.