ADP-ribosyl cyclase coupled with receptors via G proteins

Haruhiro Higashida*

Department of Biophysics, NIRI, Kanazawa University School of Medicine, 13-1 Takara-machi, Kanazawa 920, Japan

Received 17 October 1997

Abstract Crude cell membranes in mammalian cells contain ADP-ribosyl cyclase, which converts NAD^+ to cyclic ADP-ribose. Acetylcholine either increases or inhibits this activity in NG108-15 neuronal cells and adrenal chromaffin cells in a muscarinic receptor subtype-specific manner. Activation or inhibition of the cyclase activity is mimicked by GTP and blocked by bacterial toxins. These findings suggest that hormone or neurotransmitter receptors utilize the direct signaling pathway to ADP-ribosyl cyclase via G proteins within cell membranes, analogous to the previously established transduction pathways to adenylyl cyclase and phospholipase Cβ.

Key words: Signal transduction; Receptor-effector coupling; Second messenger formation; NAD^+ metabolism

Cyclic adenosine diphosphate ribose (cADPR) [1] is synthesized from β-NAD^+, an abundant intracellular substrate, by ADP-ribosyl cyclase in sea urchin eggs [2,3], ovotestis of *Aplysia* [4], canine spleen [5], pancreatic islet cells [6], murine [7] and human [8] T-lymphocytes, opossum kidney renal epithelial cells [9], bovine adrenal chromaffin cells [10], rat cardiac muscle cells [11] and NG108-15 neuronal cells [12]. Pharmacological studies suggest that cADPR is an endogenous modulator of Ca^{2+}-induced Ca^{2+} release [13,14] from ryanodine-sensitive Ca^{2+} stores [15,16], where calmodulin is an activator [17]. However, the question whether or not cADPR mediates the intracellular action of conventional hormones and neurotransmitters in mammalian cells remains unsolved. For cADPR to be accepted as an intracellular second messenger downstream of such receptors, in addition to its pharmacological effects, several biochemical criteria should be fulfilled: (1) extracellular stimulation should activate (or inhibit) ADP-ribosyl cyclase; (2) a transient decrease in substrate (NAD^+) concentrations and an increase in the product (cADPR) should occur in association; and (3) quick elimination of cADPR by cADPR hydrolase.

In sea urchin eggs, the precursor labeling protocol reveals that the formation of cADPR is enhanced by nitric oxide (NO) and cyclic GMP [3,18]. Since it is well known that NO and cyclic GMP are formed by stimulation of many kinds of agonists, it is speculated that the cADPR level is controlled by receptors through a complex cascade for the activation of ADP-ribosyl cyclase [19]. However, the exact signal pathway from hormone and neurotransmitter receptors to ADP-ribosyl cyclase in mammalian cells is still not clearly understood.

The typical pathway in mammalian cells consists of signals from receptors involved in forming intracellular second messengers, such as cyclic AMP or inositol-1,4,5-trisphosphate and diacylglycerol, being transduced to effector enzymes of adenylyl cyclase or phospholipase Cβ via G proteins in the cell surface membrane [20]. Therefore, an analogy to this established signaling pathway leads to an interesting hypothesis, namely that cADPR formation is regulated by ADP-ribosyl cyclase through the direct action of G proteins in the cell membrane. There are in fact two reports which support this hypothesis.

Stimulation of muscarinic acetylcholine receptors (mAChRs) with acetylcholine or carbamoylcholine (CCh) activates cADPR formation in bovine adrenal chromaffin cells [10] and NG108-15 neuroblastoma×glioma hybrid cells [12]. In both cases, the enzyme activity was found in crude membrane fractions. Interestingly, activation of ADP-ribosyl cyclase by CCh was observed in membranes obtained from NG108-15 cells overexpressing m1 or m3 mAChRs, while inhibition was mediated by endogenous m4 mAChRs and exogenous m2 mAChRs. These effects were mimicked by GTP in NG108-15 cells. The CCh-induced activation was inhibited by prior treatment of cells with cholera toxin, while the inhibition of ADP-ribosyl cyclase was sensitive to pertussis toxin. Although further validation is necessary to determine exactly which G proteins are involved in the stimulatory and inhibitory pathways to ADP-ribosyl cyclase, it can be hypothesized from these observations that the signal to ADP-ribosyl cyclase from mAChRs is mediated via G proteins, as shown in Fig. 1.

This signaling seems to be comparable to that from other endogenous receptors expressed in NG108-15 cells, namely α_{B2} adrenergic, δ opioid, B_2 bradykinin and P_{2u} ATP receptors, which have been shown to activate or inhibit adenylyl cyclase or to stimulate phospholipase Cβ via G proteins [21,22] (Fig. 1).

Another consequence of receptor-mediated regulation of ADP-ribosyl cyclase activity may be that the augmented or reduced cADPR may interact on the type 2 ryanodine receptors in conjunction with the augmented cytosolic Ca^{2+} resulting from Ca^{2+} influx through voltage-activated Ca^{2+} channels [23,24], and subsequently modulate intracellular Ca^{2+} concentrations.

The membrane-bound form of mammalian ADP-ribosyl cyclase is CD38, a cell surface antigen [25,26], or BST-1 [27]. CD38 contains multiple enzyme activities, and catalyzes the conversion of NAD^+ into cADPR by cyclase activity [7,28], of cADPR into ADPR by hydrolase activity [28,29], and of NAD^+ into ADPR directly by glycohydrolase activity [30,31]. This multifunctional catalytic domain of CD38 seems to be located in the extracellular N-terminal region [29,30,32]. Thus, this estimate of the membrane topology of ectoenzyme CD38 runs counter to our speculation that the catalytic site of ADP-ribosyl cyclase is in the cell interior. Therefore, a new question has arisen, whether CD38 cell surface antigen repre-

*Fax: (81) (76) 234-4236.
E-mail: Haruhiro@med.kanazawa-u.ac.jp

PII S0014-5793(97)01410-5

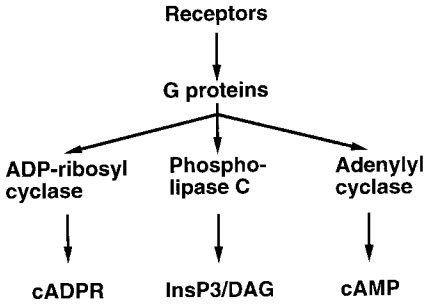

Fig. 1. Scheme of signal transduction from receptors to ADP-ribosyl cyclase, adenylyl cyclase, and phospholipase C via G proteins to produce second messengers, cADPR, cyclic AMP (cAMP), inositol trisphosphate (InsP3) and diacylglycerol (DAG), in NG108-15 cells.

sents the endogenous ADP-ribosyl cyclase in NG108-15 or in chromaffin cells.

References

[1] Lee, H.C., Aarhus, R. and Levitt, D. (1994) Nature Struct. Biol. 1, 143–144.
[2] Rusinko, N. and Lee, H.C. (1989) J. Biol. Chem. 264, 11725–11731.
[3] Galione, A., White, A., Willmott, N., Turner, M., Potter, B.V.L. and Watson, S.P. (1993) Nature 365, 456–459.
[4] Lee, H.C. and Aarhus, R. (1991) Cell Regul. 2, 203–209.
[5] Kim, H., Jacobson, E.L. and Jacobson, M.K. (1993) Science 261, 1330–1333.
[6] Kato, I., Takasawa, S., Akabane, A., Tanaka, O., Abe, H., Takamura, T., Suzuki, Y., Nata, K., Yonekura, H., Yoshimoto, T. and Okamoto, H. (1995) J. Biol. Chem. 270, 30045–30050.
[7] Howard, M., Grimaldi, J.C., Bazan, J.F., Lund, F.E., Santos-Argumedo, L., Parkhouse, R.M.E., Walseth, T.F. and Lee, H.C. (1993) Science 262, 1056–1059.
[8] Hara-Yokoyama, M., Kukimoto, I., Nishina, H., Kontani, K., Hirabayashi, Y., Irie, F., Sugiya, H., Furuyama, S. and Katada, T. (1996) J. Biol. Chem. 271, 12951–12955.
[9] Beers, K.W., Chini, E.N., Lee, H.C. and Dousa, T.P. (1995) Am. J. Physiol. 268, C741–C746.
[10] Morita, K., Kitayama, S. and Dohi, T. (1997) J. Biol. Chem. 272, 21002–21009.
[11] Mészáros, L.G., Wrenn, R.W. and Váradi, G. (1997) Biochem. Biophys. Res. Commun. 234, 252–256.
[12] Higashida, H., Yokoyama, S., Hashii, M., Taketo, M., Higashida, M., Takayasu, T., Ohshima, T., Takasawa, S., Okamoto, H. and Noda, M. (1997) J. Biol. Chem. (in press).
[13] Galione, A., Lee, H.C. and Busa, W.B. (1991) Science 253, 1143–1146.
[14] Guo, X. and Becker, P.L. (1997) J. Biol. Chem. 272, 16984–16989.
[15] Sitsapesan, R., McGarry, S.J. and Williams, A.J. (1995) Trends Pharmacol. Sci. 16, 386–391.
[16] Mészáros, L.G., Bak, J. and Chu, A. (1993) Nature 364, 76–79.
[17] Lee, H.C., Aarhus, R., Graeff, R., Gurnack, M.E. and Walseth, T.F. (1994) Nature 370, 307–314.
[18] Willmott, N., Sethi, J.K., Walseth, T.F., Lee, H.C., White, A.M. and Galione, A. (1996) J. Biol. Chem. 271, 3699–3705.
[19] Berridge, M.J. (1993) Nature 365, 388–389.
[20] Gilman, A.G. (1984) Cell 36, 577–579.
[21] Noda, M., Ishizaka, N., Yokoyama, S., Hoshi, N., Kimura, Y., Hashii, M., Taketo, M., Egorova, A., Knijnik, R., Fukuda, K., Morikawa, H., Brown, D.A. and Higashida, H. (1996) J. Lipid Mediator Cell Signal. 14, 175–185.
[22] Higashida, H., Hashii, M., Yokoyama, S., Taketo, M., Hoshi, N., Noda, M., Zhong, Z.-G., Shahidullah, M., Minabe, Y., Nakashima, S. and Nozawa, Y. (1996) Prog. Brain Res. 113, 215–230.
[23] Ying-Hua, S., Tokimasa, T., Takasawa, S., Furuya, Y., Nohmi, M., Okamoto, H. and Kuba, K. (1994) Neuron 12, 1073–1079.
[24] Empson, R.M. and Galion, A. (1997) J. Biol. Chem. 272, 20967–20970.
[25] Harada, N., Santos-Argumedo, L., Chang, R., Grimaldi, J.C., Lund, F.E., Brannan, C.I., Copeland, N.G., Jenkins, N.A., Heath, A.W., Parkhouse, R.M.E. and Howard, M. (1993) J. Immunol. 151, 3111–3118.
[26] Lund, F., Solvason, N., Grimaldi, J.C., Parkhouse, R.M.E. and Howard, M. (1995) Immunol. Today 16.
[27] Hirata, Y., Kimura, N., Sato, K., Ohsugi, Y., Takasawa, S., Okamoto, H., Ishikawa, J., Kaisho, T., Ishihara, K. and Hirano, T. (1994) FEBS Lett. 356, 244–248.
[28] Takasawa, S., Tohgo, A., Noguchi, N., Koguma, T., Nata, K., Sugimoto, T., Yonekura, H. and Okamoto, H. (1993) J. Biol. Chem. 268, 26052–26054.
[29] Tohgo, A., Takasawa, S., Noguchi, N., Koguma, T., Nata, K., Sugimoto, T., Furuya, Y., Yonekura, H. and Okamoto, H. (1994) J. Biol. Chem. 269, 28555–28557.
[30] Hoshino, S., Kukimoto, I., Kontani, K., Inoue, S., Kanda, Y., Malavasi, F. and Katada, T. (1997) J. Immunol. 158, 741–747.
[31] Umar, S., Malavasi, F. and Mehta, K. (1996) J. Biol. Chem. 271, 15922–15927.
[32] Prasad, G.S., McRee, D.E., Stura, E.A., Levitt, D.G., Lee, H.C. and Stout, C.D. (1996) Nature Struct. Biol. 3, 957–964.