Volume 304, number 1, 9–11 FEBS 11101 June 1992

Relationship of neural cell adhesion molecules (N-CAMs) with adenylate cyclase

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Received 22 January 1992; revised version received 3 April 1992

Bovine brain cDNA cloned earlier and attributed to calmodulin-independent adenylate cyclase encodes the neural cell adhesion molecule (N-CAM). The expression of N-CAM mRNA in *Xenopus laevis* oocytes increases their basal adenylate cyclase activity. Polyclonal antibodies against synthetic peptide A, VAENQQGKSKAAHFV (664–678 amino acid residues of bovine N-CAM), containing sequence AXXXXGKS which is homologous to the nucleotide-binding consensus sequence GXXXXGKS, inhibit the adenylate cyclase activity. A close relationship appears to exist between adenylate cyclase and N-CAM.

Adenylate cyclase; Neural cell adhesion molecule

1. INTRODUCTION

Recently we published data on the cloning of a cDNA which was believed to encode calmodulin-independent adenylate cyclase from bovine brain [1]. The protein was isolated by chromatography of the Lubrol-soluble fraction of bovine cortex membranes on the immunosorbent fraction with monoclonal antibody Mab-1 [2]. The antibody impeded the enzymatic activity of adenylate cyclase and adsorbed the enzyme activity after immobilization on protein A–Sepharose. The preparation eluted from the immunosorbent fraction by conventional methods had not retained any adenylate cyclase activity, however, the eluate displayed slight adenylate cyclase activity after reduction of the disulfide bonds of the immunosorbent immunoglobulins [2]. Polyclonal antibodies to the isolated protein effectively inhibited the adenylate cyclase activity, and, after immobilization, they also decreased the enzyme activity in solution [2]. Comparison of our cDNA sequence with sequences in the GenBank database, performed by R.T. Premont, showed a high similarity between this sequence and those of rat, human and mouse neural cell adhesion molecule (N-CAM) cDNA [3]. Analysis of the PIR database revealed similar data. The cDNA we cloned could indeed be attributed to that of N-CAM from bovine brain. The results described here, and those obtained earlier, indicate a close relationship between adenylate cyclase and N-CAM.

Abbreviations: N-CAM, neural cell adhesion molecule; γPDE, γ-subunit of cGMP phosphodiesterase; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay.

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2. MATERIALS AND METHODS

2.1. *Expression in oocytes*

pSP65 plasmid containing the full-length N-CAM cDNA was linearised and used for in vitro transcription with SP6 RNA polymerase in the presence of GppG [4,5]. The N-CAM mRNA (6–50 ng) in water (50 nl) was microinjected into collagenase-treated *Xenopus laevis* prepared as in [6]. Fifty oocytes from each region were homogenized 24 h later, and the adenylate cyclase activity of the homogenate was measured. Oocytes injected with water (50 nl/oocyte) and γPDE mRNA (25 ng/oocyte) were used as controls.

2.2. *Brain cortex membranes*

Brain cortex membranes were isolated according to [7] and the membrane proteins were solubilized with 1% Lubrol PX as in [2]. The preparation of calmodulin-independent adenylate cyclase was obtained by chromatography on DEAE-Sephacell and calmodulin–Sepharose as described in [2].

2.3. *Adenylate cyclase activity*

Adenylate cyclase activity was determined according to [8]. The influence of the antibodies on adenylate cyclase activity and immunoprecipitation were studied as in [2].

2.4. *Obtaining antibodies to peptide A*

Rabbits were immunized with synthetic peptide A conjugated with BSA by means of glutaric aldehyde [9]. The animals were injected every 14 days. The conjugate peptide A-BSA (200 mkg) in physiological salt solution (0.4 ml) was used for one immunization. The first injection was done in a mixture with complete Freund's adjuvant, and all subsequent ones were performed with incomplete Freund's adjuvant. Blood was taken on the 12th day after the fifth immunization. The immunoglobulins were purified by salting-out with a semi-saturated solution of ammonium sulphate and subsequent chromatography on DE-52 (Wattman). The titre of antibodies was determined according to the ELISA method.

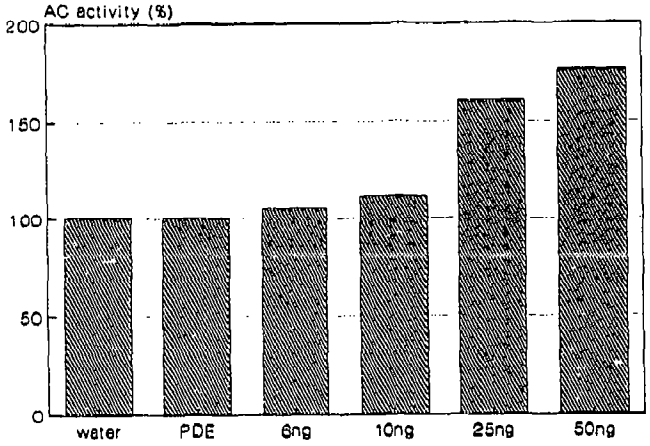

Fig. 1. Dependence of adenylate cyclase (AC) activity of *Xenopus laevis* oocytes on the amount of injected N-CAM mRNA.

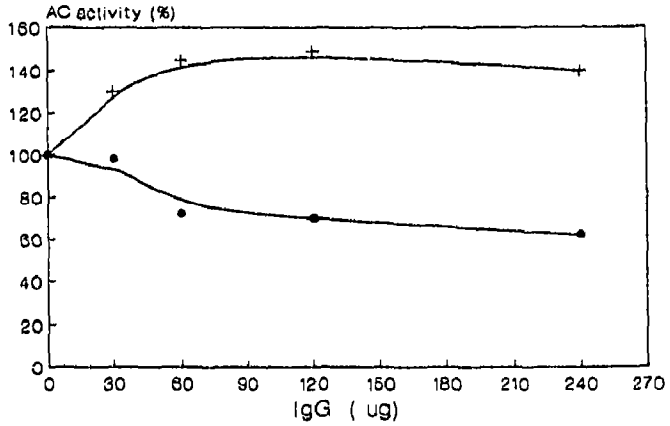

Fig. 2. Influence of antibodies on the adenylate cyclase (AC) activity of the soluble fraction of brain cortex membranes. (+) Normal IgG of rabbit; (■) antibodies to peptide A.

3. RESULTS AND DISCUSSION

Comparison of our cDNA sequence (presumed to code for calmodulin-independent adenylate cyclase from bovine brain) [1] and the sequences in the PIR database (release 21) (conducted in 1990) revealed a high homology between our sequence and those of the human, rat and mouse N-CAMs. Thus we, as well as R.T. Premont [3], concluded that a cDNA of N-CAM from bovine brain had been cloned. There are several possible explanations for these two observations: (i) adenylate cyclase and N-CAM-like activities belong to the same molecule; (ii) N-CAM and adenylate cyclase have identical antigenic determinants and the monoclonal antibodies interact with the two proteins; (iii) adenylate cyclase has a high affinity to N-CAM and the proteins had been co-purified; (iv) N-CAM is capable of activating adenylate cyclase directly or when interacting with other proteins.

To choose between these hypotheses we expressed N-CAM in various systems to find the adenylate cyclase activity of the protein: negative results were obtained for *E. coli* and in vitro systems (data not shown). However, injection of N-CAM mRNA into *Xenopus laevis* oocytes increased the adenylate cyclase activity. Moreover, the activity was dependent on the amount of mRNA (Fig. 1).

Polyclonal antibodies were obtained to synthetic peptide A VAENQQGKSKAAHFV (664–678 amino acid residues of bovine N-CAM). The peptide included the sequence AXXXXGKS (665–672), which is homologous to the nucleotide-binding consensus sequence GXXXXGKS [10] located in the catalytic sites of bacterial adenylate cyclases [11] but absent in the cloned mammalian adenylate cyclases of type I [12] and type III [13]. According to ELISA experiments the antibodies interacted with purified N-CAM (data not shown). The antibodies, as compared to the normal rabbit immunoglobulins, effectively inhibited adenylate cyclase activity of the soluble fraction of brain cortex membranes (Fig. 2). When immobilized on protein A–Sepharose, the antibodies again decreased the enzyme activity of the soluble fraction (Fig. 3). Moreover, treatment of the partially purified preparation of calmodulin-independent adenylate cyclase (lacking a calmodulin-sensitive enzyme) by the antibodies lowered the enzyme activity almost completely (Fig. 4).

Interestingly, a novel type of brain synaptosomal adenylate cyclase has lately been characterized by monoclonal antibodies to the synthetic peptide B (GVATKGLNVHGKSSDWG), corresponding to amino acid sequence 342–358 of the *Bacillus anthracis* adenylate cyclase and containing consensus sequence GXXXXGKS [14]. We believe that the above data testify to a direct relationship between N-CAM and adenylate cyclase. The data do not contradict the con-

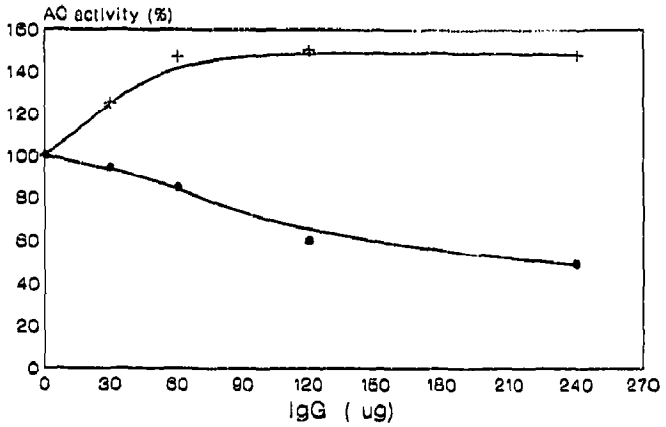

Fig. 3. Adenylate cyclase (AC) activity of the soluble fraction of brain cortex membranes after immunoprecipitation with antibodies immobilized on protein A–Sepharose. (+) Normal IgG of rabbit; (■) antibodies to peptide A.

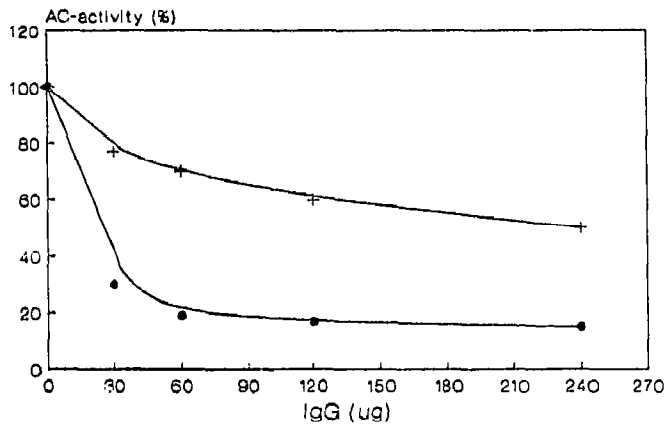

Fig. 4. Influence of antibodies on the adenylate cyclase (AC) activity of the calmodulin-independent enzyme preparation. (+) Normal IgG of rabbit; (■) antibodies to peptide A.

cept that N-CAM activates adenylate cyclase: precipitation of N-CAM by antibodies eliminates this effect. The major component of N-CAMs (N-CAM-180) appears to interact with the cytoskeleton via the membrane-bound protein, spectrin [15]. Antibodies to spectrin (fodrin) are able to precipitate the adenylate cyclase activity (D. Storm, unpublished observation). Besides, the hypothesis [16] claims that N-CAM influences the adenylate cyclase inhibiting protein, G_i.

Acknowledgements: We are particularly grateful to Igor L. Rodionov for synthesis of peptide A, and to Alexandre K. Philippov for his help on the work with oocytes. We are thankful to Tatyana E. Chernichko for translation and to Nikolai V. Khramtsov for helpful discussions.

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