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Review

Adenylyl cyclases in the digestive system

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ABSTRACT

Adenylyl cyclases (ACs) are a group of widely distributed enzymes whose functions are very diverse. There are nine known transmembrane AC isoforms activated by Gαs. Each has its own pattern of expression in the digestive system and differential regulation of function by Ca^{2+} and other intracellular signals. In addition to the transmembrane isoforms, one AC is soluble and exhibits distinct regulation. In this review, the basic structure, regulation and physiological roles of ACs in the digestive system are discussed.

Contents

Abbreviations: AC, adenylyl cyclase; CCK, cholecystokinin; CFTR, cystic fibrosis transmembrane regulator; CREBs, cAMP response element binding proteins; EGF, epidermal growth factor; Epac, Exchange protein directly activated by cAMP; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GPCR, G protein coupled receptors; GTP, guanosine triphosphate; NKCC, Na^+–K^+–Cl^- cotransporter; NO, nitric oxide; PKA, cAMP-dependent protein kinase A; PKC, protein kinase C; TNF-α, tumor necrosis factor-α; VIP, vasoactive intestinal polypeptide.

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1. Introduction

Adenylyl cyclases (ACs) catalyze the conversion of ATP to cAMP and pyrophosphate. The reaction occurs in a single step where the oxygen on 3′hydroxyl group of ATP nucleophilically attacks the α-phosphate forming a phosphodiester bond and cleaving a pyrophosphate group [1]. Once generated, cAMP acts as a second messenger through two primary pathways: (1) by promoting proteins phosphorylation by activation of cAMP-dependent protein kinase (PKA) or (2) by activating exchange protein directly activated by cAMP (Epac). PKA is a heterotetramer composed of two subunits: a catalytic subunit that contains the enzyme's active site, and an ATP-binding domain, as well as a regulatory subunit that binds cAMP [2]. PKA phosphorylates a wide range of proteins at serine and threonine residues. The targets can be enzymes such as phosphorylase kinase, ion channels including cystic fibrosis transmembrane regulator (CFTR), chromosomal proteins such as histone H1, and transcription factors including cAMP response element binding proteins (CREBs) [3]. In rat pancreatic acini, PKA induces a potentiation of Ca^{2+}-dependent amylase secretion [4]. In dispersed rat submandibular and parotid cells, PKA triggers exocytosis and phosphorylates a 26-kDa integral membrane protein [5]. CREB phosphorylation is observed upon PKA activation in both parotid acini [6] and pancreatic acini [7,8]. Epac, like PKA, has two subunits: a catalytic subunit that contains a CDC25-homology domain, a REM (Ras Exchange Motif) and a RA (Ras Association) domain, as well as a regulatory subunit that binds cAMP [9]. Epac is a guanine nucleotide exchange factor (GEF) for the small G proteins Rap1 and Rap2. In both pancreatic acini and parotid acini, Epac activation leads to increased amylase secretion [7,10,11].

Ten AC isoforms have been identified: nine isoforms are transmembrane and activated by Gαs. The single "soluble" AC10 isoform that lacks the transmembrane domains, is insensitive to Gαs, and more closely resembles the cyanobacterial AC enzymes than the transmembrane ACs [12].

The functions of ACs in the heart, kidney and brain have been well described, but are lacking for digestive tract [13–15]. In this manuscript, we provide a comprehensive review of the structure of a single AC, the regulation of AC isoforms, as well as their physiological and pathophysiological roles in the digestive system.

2. Structure of adenylyl cyclase

At least 9 transmembrane AC isoforms (AC1, AC2, AC3, AC4, AC5, AC6, AC7, AC8 and AC9), two splice variants of AC8 and one soluble AC isoform (AC10) have been cloned and characterized in mammals.

The transmembrane AC isoforms share a large sequence homology in the primary structure and similar predicted three-dimensional structure. Each transmembrane AC isoform is coded by a different gene located in a different chromosome, with the exception of humans which have two genes that encode AC7 and AC9 on chromosome 16 [16]. AC structure can be divided in five major domains: 1) the NH2 terminus, 2) the first transmembrane cluster (TM1), 3) the first cytoplasmic loop composed of C1a and C1b, 4) the second transmembrane cluster (TM2) with extracellular N-glycosylation sites, and 5) the second cytoplasmic loop composed of C2a and C2b (Fig. 1). The transmembrane regions are composed of six membrane-spanning helices, which cross the plasma membrane 12 times in 2 clusters of 6 TM domains (TM1 and TM2), whose function is to keep the enzyme anchored in the membrane. The cytoplasmic regions C1 and C2 are approximately 40 kDa each and can be further subdivided into C_{1a}, C_{1b}, C_{2a}, and C_{2b}. Both C1a and C2a are highly conserved catalytic ATP-binding regions [17], which dimerize to form a pseudosymmetric enzyme, which forms the catalytic site. ATP binds at one of two pseudosymmetric binding sites of the C1–C2 interface. A second C1 domain subsite includes a P-loop that accommodates the nucleotide phosphates and two conserved acid residues that bind to ATP through interaction with two Mg^{2+}; one Mg^{2+} contributes to catalysis, whereas the second one interacts with nucleotide β- and γ-phosphates from substrate binding and possibly for leaving group stabilization. Both C2a and C2b are less conserved than the C1 domain [17,18]. These two cytoplasmic regions are responsible for regulation by the G-proteins subunits α and βγ and specific intracellular signals [19]. The C_{1b} domain is the largest domain and contains several regulatory sites, and has a variable structure across the isoforms. The C_{2b} domain is essentially non-existent in many isoforms, and has not yet been associated with a function [20].

The soluble AC is the most divergent in its sequence and is similar to ACs found in cyanobacteria. Soluble AC was originally isolated from the testis [12,21,22] and has been found in other tissues such as pancreas [23–25]. There are several splice variants but the two resulting in the

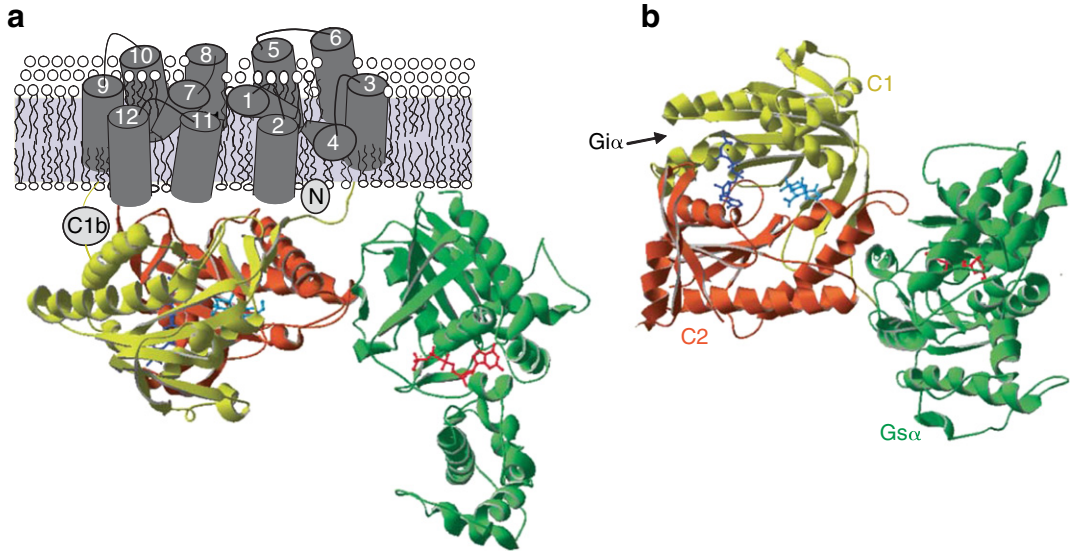

Fig. 1. Crystal structure of adenylyl cyclase. a) The figure shows the catalytic domains of mammalian AC (C1 and C2) with Gαs (green) and Gαi. The location of forskolin (cyan) and P-site inhibitor (dark blue) is also appreciated. b) An alternate view from cytoplasmic side, showing forskolin and catalytic site. The interaction site of Giα with C1 domain is indicated by an arrow. This figure was obtained with permission from [13].