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Membrane-Bound Atp-Dependent Energy System (A Short Historic View from Their Discoveries up to Now)

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Additional information is available at the end of the chapter

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This chapter is important and hence we felt that some general information has to be given globally on the physiological research, as we could not do a detailed study being interns in the medical department of the medical services.

We met the presentations of these new results at different times in our life, which modified our aims and possibilities in the research. On the other hand, our lives gave to us a possibility to our scientific and methological knowledges to incorporate these new fields in our research field.

We have to learn the following:

1. Being clinicians, our main work is related to the medical service of patients' problems; however, we wanted to learn the main biochemical events in the gastrointestinal tract (in terms of development of mucosal damage and prevention);
2. We started with the biochemical examinations of gastric tissues from 1960s, and these new observations offered us to do the forthcoming steps in our research work;
3. We had time periods in our lives when we had contaminating (infecting) with basic and fruitful international research lines, when we had opportunities to participate and to work in research of different foreign countries (GyM in Norway in 1968–1969 and USA in 1985; IL Szabo in USA in 1999–2001);
4. We learned new research methods while working with foreign countries, and we tried to apply the new knowledge in researches that we did in our country (Hungary);
5. It was very clear that there is a big gap between the problems (results) of a basic research and clinical practice; however, we wanted to understand the main common problems in the basic research and in clinical science;

6. We had many ethical and medical problems in doing biochemical observations in the gastrointestinal resectates of human GI tract in patients with peptic ulcer who underwent surgical interventions owing to their primary disease;
7. We participated in the different international forums of GI research (as established persons, future leaders, organizers and responsible persons).

Our main lines of research series of international conferences are as follows:

- a. An international series of international conferences [International Conference of Experimental Ulcer (from 1970 to 1994), International Conference on Ulcer Research (from 1994 to 2000) and International Conference on Gastrointestinal Research (from 2000)] has been internationally established at Copenhagen (Denmark) – in connection with Fourth World Congress of Gastroenterology – in 1970. Thereafter, different international conferences were organized in different countries of different continents (Copenhagen, Denmark, 1970; Cologne, Germany, 1972; Parádördő, Hungary, 1976; Tokyo, Japan, 1980; Boston, USA, 1985; Jerusalem, Israel, 1988; Berlin, Germany, 1991; Kyoto, Japan, 1994; Hong Kong, China, 1997; Budapest-Pécs, Hungary, 2000; Dubrovnik, Croatia, 2003; Osaka, Japan, 2006; Split, Croatia, 2009; Tokyo, Japan, 2012) (for details, see Mózsik, 2006b);
- b. Another series of international symposiums was established in connection with main World Congresses of the International Union of Pharmacology (from 1984). The gastrointestinal section of International Union of Pharmacology was established at Montreal (Canada) in 1994 (IUPHAR GI Section); however, this series of the international symposiums started earlier (Pécs, Hungary, 1984; Pécs, Hungary, 1990; Pécs, Hungary, 1995; Sperlonga, Italy, 1996; Pécs, Hungary, 1998; Honolulu, USA, 2002; Kyoto, Japan, 2004; Osaka, Japan, 2006; Honolulu, Hawaii, USA, 2009; Tokyo, Japan, 2012) (for details, see Mózsik et al., 2006);
- c. International Symposia on “Cell/Tissue Injury and Cytoprotection/Organoprotection” [Heidelberg, Germany, 1986; Boston, USA, 1989; Long Beach, CA, USA, 2000; 2006; Yalta (Crimea), Ukraine, 2008; Saint Petersburg, Russia, 2011; Honolulu, USA, 2012; Budapest, Hungary, 2014];
- d. International Symposia on Gastrointestinal Cytoprotection (Pécs, Hungary, 1983; 1987; 1991; 1995, 2000);
- e. International Symposia of the “International Brain-Gut Society” (Lake Arrowhead, Los Angeles, USA, 1988; Pécs, Hungary, 1990; Florence, Italy, 1993; Pécs, Hungary, 1995);
- f. Interscience World Conferences on Inflammation [Antirheumatics, Analgesics, Immunomodulators (Venice, Italy, 1984; Monte Carlo, France, 1986; 1989; Geneva, Switzerland, 1995; 1998)].
8. This part of the book has been tried to give a short information on the different membrane-bound ATP-dependent energy systems with emphasizing the main problems in these

fields, however in briefly up to now (we had no these summaries of these studies in our mind during the time of our observations done in GI tract).

We have to emphasize that we have not done a very detailed study on the membrane-bound ATP-dependent energy systems; however, the results of our study (namely the study of the details of ATP splitting by different membrane-bound enzymes, Na^+ - K^+ -dependent ATPase and adenylate cyclase, represent different pathways of energy-liberating cellular events) offered us a very successful research possibility in our field. As ATP splitting results in energy liberation of cells, and the circumstances of the ATP resynthesis (by oxidative phosphorylation) confirmed the presence of tissue hypoxia in the GI mucosa under different (e.g., in the ulcerated or the damaged mucosal tissues) experimental and clinical circumstances.

The main lines of the studies on membrane-bound ATP-dependent energy systems were carried dominantly from GI tract, except for H^+ - K^+ -ATPase system (we did not participate in these studies), and hence we started with these studies in our field. Furthermore, these studies offered good possibility to understand the presence of tissue hypoxia (anoxia) during the development of mucosal damage, which was suggested as one of the main reasons (or consequences) of peptic ulcer.

Before the demonstrations of our biochemical results, we will give a very short summary of the main membrane-bound ATP-dependent energy systems (of course, not in the gastric mucosa).

5.1. Na^+ - K^+ -dependent ATP-ase

This enzyme (other names: sodium–potassium adenosine triphosphatase, Na^+/K^+ pump, sodium–potassium pump, sodium pump) is located in the plasma membrane of all animal cells. The Na^+ - K^+ -ATPase enzyme pumps sodium out of the cells, while pumping potassium into the cells.

Active transport is responsible for cells containing relatively high concentrations of potassium ions but low concentrations of sodium ions. The mechanism responsible for this sodium–potassium pump moves these two ions in opposite directions across the plasma membrane. This was investigated by following the passage of radioactive ions across the plasma membrane of certain cells. It was found that the concentrations of sodium and potassium ions on the two sides of the membrane are independent, which suggests that the same carrier transports both ions. It is now known that the carrier is an ATPase and it pumps three sodium ions of the cell for every two potassium ions pumped in.

The sodium–potassium pump was discovered in the 1950s (Skou, 1957) by Jens Christian Skou (Department of Physiology, Aarhus University, Denmark), who was awarded Nobel Prize in chemistry in 1997 (<http://nobelprize.org/chemistry/laureates/1997/index.html>). It marked an important step forward in our understanding of how ions get into and out of cells, and it is particularly significant for excitable cells such as nervous cells, which depend on this pump for responding to stimuli and transmitting impulses (Skou, 1965).

5.1.1. Schematic morphological bases of sodium-potassium pump ($\text{Na}^+\text{-K}^+$ -dependent adenosine triphosphatase enzyme)

The $\text{Na}^+\text{-K}^+$ -ATPases belong to the P_2 class and specifically to the P_{2c} subclass of ATPases. These ATPases are composed of two subunits (α and β). The α -subunit (about 113 kD) binds ATP and both Na^+ and K^+ ions and contains the phosphorylation sites typical of the P-type ATPases. The autophosphorylation site is the P domain. The P-type ATPases are also subject to additional phosphorylation events via other kinases. The smaller β -subunit (about 35-kD glycoprotein) is absolutely necessary for activity of the complex. It appears to be critical in facilitating the plasma membrane localization and activation of the α -subunit.

Several isoforms of both α - and β -subunits have been identified that exhibit different kinetic parameters and tissue distribution. There are four α -subunit genes and three β -subunit genes in humans. The α_1 isoform is the predominant form and is ubiquitously expressed. The α_2 isoform is primarily expressed in muscle tissues (skeletal, smooth and cardiac) as well as in adipose tissue, brain and lungs. The α_3 isoform is expressed primarily in the heart and neurons. The α_4 isoform is only expressed in the testes. The β_1 isoform is ubiquitously expressed and is associated with the α -subunit in the ubiquitously expressed $\alpha_1 \beta_1 \text{Na}^+\text{-K}^+$ -ATPase complex. There are many more detailed parts and interactions of $\text{Na}^+\text{-K}^+$ -ATPase discovered and proved in the last decades (<http://themedicalbiochemistrypage.org/membranes.php>; Morth et al., www.nature.com/reviews/molcellbio 12:60–70, 2011).

5.1.2. Main functions of $\text{Na}^+\text{-K}^+$ -ATPase

The $\text{Na}^+\text{-K}^+$ -ATPases help to maintain resting potential and regulate cellular volume (Hall and Guyton, 2006). It also functions as signal transducer/integrator to regulate the mitogen-activated protein kinase (MARK) pathway, mitochondrial reactive oxygen species (ROS) as well as intracellular calcium. In most animal cells, the $\text{Na}^+\text{-K}^+$ -ATPase is responsible for about 1/5 of the cell's energy expenditure, meanwhile, for neurons, the $\text{Na}^+\text{-K}^+$ -ATPase can be responsible for up to 2/3 of the energy expenditure (Howard et al., 2012).

5.1.2.1. Resting potential

In order to maintain the cell membrane potential, cells keep a low concentration of sodium ions and high levels of potassium within the cell (intracellular). The sodium–potassium pump moves three sodium ions out and moves two potassium ions in, thus in total removing one positive-charge carrier from the intracellular space.

5.1.2.2. Transport

Export of sodium from the cell provides the driving force for several secondary active transporters, membrane transport proteins, which import glucose, amino acids and other nutrients into the cell by the use of sodium gradient.

Another important task of the $\text{Na}^+\text{-K}^+$ -pump is to provide a Na^+ gradient that is used by certain carrier processes. In the gut, sodium is transported out of the reabsorbing cell on the blood

(intestinal fluid) side via the $\text{Na}^+\text{-K}^+$ -pump, whereas, on the reabsorbing (luminal) side, the Na^+ -glucose symporter uses the created Na^+ gradient as a source of energy to import both Na^+ and glucose, which is far more efficient than simple diffusion. Similar processes can be found and located in the renal tubular system.

5.1.2.3. Controlling cell volume

Failure of the $\text{Na}^+\text{-K}^+$ pumps can result in swelling of the cell. A cell's osmolarity is the sum of the concentrations of the various ion species and many proteins and other organic compounds inside the cell. When this is higher than the osmolarity outside of the cell, water flows into the cell through osmosis. This can cause the cell to swell up and lyse. The $\text{Na}^+\text{-K}^+$ pump helps to maintain the right concentrations of ions. Furthermore, when the cell begins to swell, this automatically activates the $\text{Na}^+\text{-K}^+$ pump.

Within the last decade, many independent laboratories have demonstrated that, in addition to classical ion transporting, this membrane protein can relay extracellular ouabain-binding signaling into the cell through regulation of protein tyrosine phosphorylation. The downstream signals through ouabain-triggered protein phosphorylation events include activation of the mitogen-activated protein kinase (MARK) signal cascades, mitochondrial reactive oxidative species (ROS) production, as well as activation of phospholipase C (PLC) and inositol triphosphate (IP_3) receptor (IP_3R) in different intracellular compartments (Yuan et al., 2005).

Protein-protein interactions play a very important role in $\text{Na}^+\text{-K}^+$ pump-mediated signal transduction (e.g., $\text{Na}^+\text{-K}^+$ pump interacts directly with Scr, a non-receptor tyrosine kinase) to form a signaling receptor complex (Tian et al., 2006). Scr kinase is inhibited by $\text{Na}^+\text{-K}^+$ pump, while, upon ouabain binding, the Scr kinase domain will be released and then activated. Based on this scenario, NaKtide, a peptide Scr inhibitor derived from the $\text{Na}^+\text{-K}^+$ pump, was developed as a functional ouabain- $\text{Na}^+\text{-K}^+$ pump-mediated signal transduction (Li et al., 2009; Forrest et al., 2012; Cannon, 2004; Calderon et al., 2011; Young et al., 2008).

5.1.3. Main steps of mechanism

The following main steps are involved in the mechanism:

- The pump, while binding ATP, binds three intracellular Na^+ ions (Hall and Guyton, 2006);
- ATP is hydrolyzed, leading to phosphorylation of the pump at a highly conserved aspartate residue and subsequent release of ADP;
- A conformational change in the pump exposes the Na^+ ions to the outside. The phosphorylated form of the pump has a low affinity for Na^+ ions, so they are released;
- The pump binds two extracellular K^+ ions. This causes the phosphorylation of the pump, reverting it to its previous state, transporting the K^+ ions into the cells;
- The unphosphorylated form of the pump has a higher affinity for Na^+ ions than K^+ ions, so two bound K^+ ions are released. ATP binds and the process starts again.

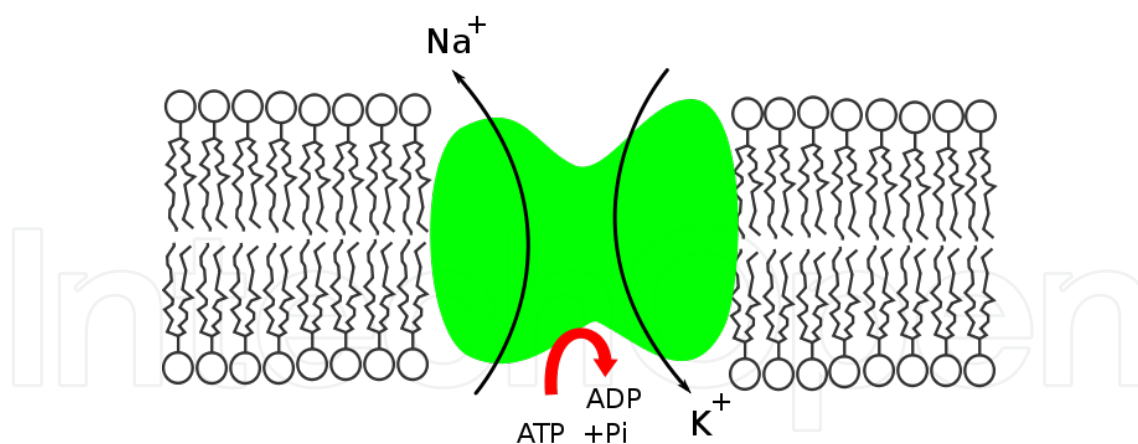


Figure 21. Schematic representation of the sodium pump ($\text{Na}^+\text{-K}^+$ ATPase): flow of ions (<http://enzyme.expasy.org/EC/3.6.3.9>).

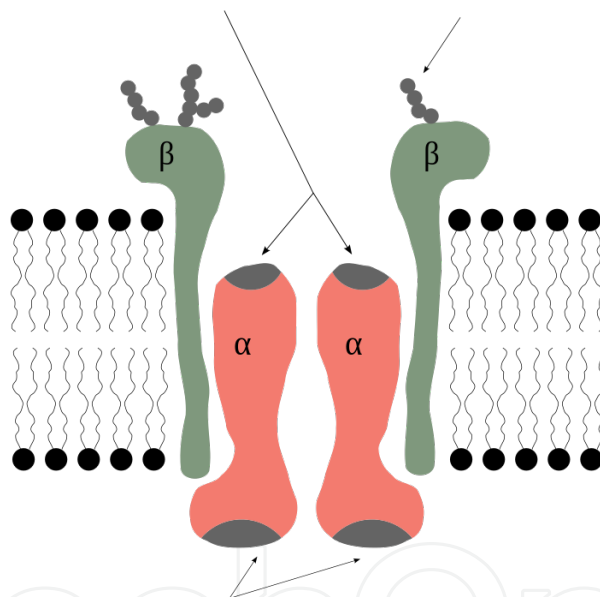


Figure 22. Schematic representation of the sodium pump ($\text{Na}^+\text{-K}^+$ ATPase): alpha and beta units (<http://enzyme.expasy.org/EC/3.6.3.9>).

The function of $\text{Na}^+\text{-K}^+$ pump can be regulated endogenously and exogenously.

The $\text{Na}^+\text{-K}^+$ pump is upregulated by cyclic adenosine monophosphate (cAMP) (Bunnier 2008). Thus, substances causing an increase in cAMP level upregulate the $\text{Na}^+\text{-K}^+$ ATPase. These include the ligands of the G_s -coupled GPCRs. In contrast, the substances causing a decrease in cAMP downregulate the $\text{Na}^+\text{-K}^+$ -ase. These include the ligands of G_i -coupled GPCRs.

The ouabain (as cardiac glycoside) inhibits the $\text{Na}^+\text{-K}^+$ ATPase activity. The prove the presence of ouabain inhibition of different preparates of plasma membranes was one of the most criteria to indicate presence a good $\text{Na}^+\text{-K}^+$ -dependent ATPase.

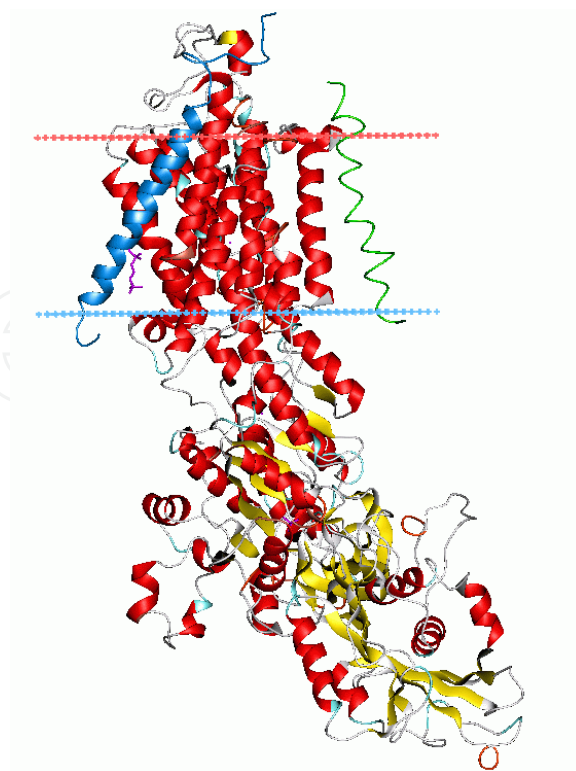


Figure 23. Schematic representation of the sodium pump ($\text{Na}^+\text{-K}^+$ ATPase), E2-P_i state. Calculated hydrocarbon boundaries of the lipid bilayer are shown as blue (intracellular) and red (extracellular) planes (<http://enzyme.expasy.org/EC/3.6.3.9>).

5.2. The gastric $\text{H}^+\text{-K}^+\text{-ATPase}$

The gastric hydrogen potassium ATPase or $\text{H}^+\text{/K}^+\text{-ATPase}$ is the proton pump of the stomach and hence it is the enzyme primarily responsible for the acidification of the stomach (acid secretion) (Potassium Hydrogen ATPase (https://www.nlm.nih.gov/cgi/mesh/2011/MB_cgi?mode=&term=Potassium+Hydrogen+ATPase)).

The $\text{H}^+\text{-K}^+\text{-ATPase}$ is found in the parietal cells, which are highly specialized epithelial cells located in the inner cell lining of the stomach called gastric mucosa. Parietal cells process an extensive secretory membrane system and the $\text{H}^+\text{-K}^+\text{-ATPase}$ is the major protein constituent of these membranes.

The enzyme that keeps the stomach at pH 0.6:

- The parietal cells of the gastric mucosa (lining of the stomach) have an external pH of 7.4;
- $\text{H}^+\text{-K}^+\text{-ATPase}$ pumps protons from these cells into the stomach (using energy of ATP) to maintain a pH difference across a single plasma membrane of 6.6;
- This is the largest known transmembrane gradient in eukaryotic cells.

5.2.1. The gastric $\text{H}^+\text{-K}^+\text{-ATPase}$ can be characterized by the followings

- $\text{H}^+\text{-K}^+\text{-ATPase}$ is similar in many respects to $\text{Na}^+\text{-K}^+\text{-ATPase}$ and $\text{Ca}^{2+}\text{-ATPase}$;

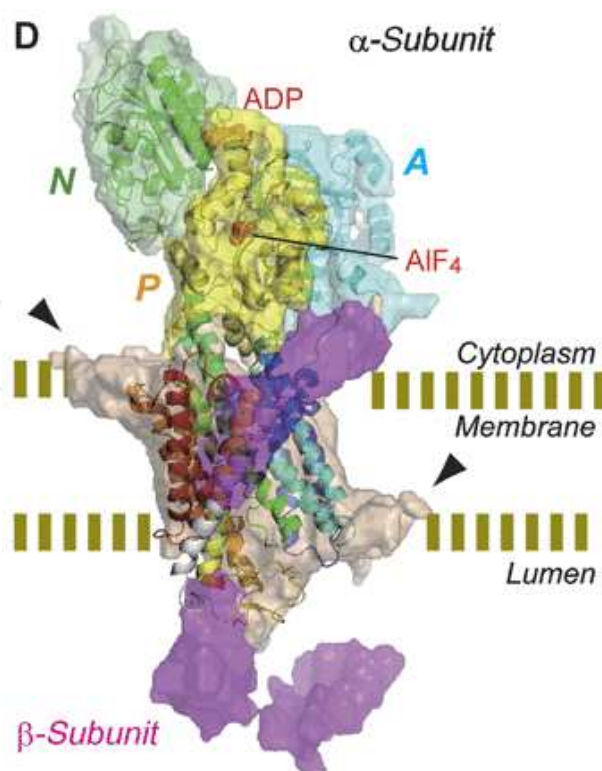


Figure 24. Schematic surface representation of gastric H^+-K^+ -ATPase $\alpha\beta$ -protomer with the fit homology model in ribbon representation. Color code of the density map: N domain, green; A domain, cyan; P domain, yellow; TM domain of the α -subunit, wheat; β -subunit, magenta. Color code of the homology model: N, A and P domains have the same color as the density map; TM helices M1–M10 of the α -subunit, gradual change from M1 (blue) to M10 (red); TM helix of β -subunit, white. The dotted lines indicate the probable position of the lipid head group and result in total thickness of approximately 35 Å, which is based on the densities protruding perpendicular from the TM domain (arrowheads). The bound ADP and AlF_4 molecules are shown as red sphere.

- All three enzymes form covalent E–P intermediates (P-type pumps);
- All three have similar sequences for the large (α) subunit;
- All three are involved in active transport.

The H^+-K^+ -ATPase is a heterodimeric protein, the product of two genes. The gene ATP4A encodes the H^+-K^+ -ATPase α -subunit and is about 1000 amino acid protein that contains the catalytic sites of enzyme and forms the pore through the cell membrane that allows the transport of ions.

The gene ATP4B encodes the β -subunit of the H^+-K^+ -ATPase, which is about 300-amino acid protein with a 36 –amino acid N-terminal cytoplasmic domain, a single transmembrane domain and a highly glycosylated extracellular domain. The H^+-K^+ -ATPase β -subunit stabilizes the H^+-K^+ -ATPase α -subunit and is required for function of the enzyme. It also appears to contain signals that direct the heterodimer to membrane destinations within the cell, although some of these signals are subordinate to signals found in H^+-K^+ -ATPase α -subunit.

The H^+-K^+ -ATPase is a member of the P-type ATPase superfamily, a large family of related proteins that transport ions, usually cations, across biological membranes of nearly all species.

The H^+ - K^+ -ATPase transports one hydrogen ion (H^+) from the cytoplasm of the parietal cell in exchange for potassium (K^+) retrieved from the gastric lumen. As an ion pump, the H^+ - K^+ -ATPase is able to transport ions against a concentration gradient using energy derived from the hydrolysis of ATP. Like all P-type ATPases, a phosphate group is transferred from adenosine triphosphate (ATP) to the H^+ - K^+ -ATPase during transport cycle. This phosphate transfer powers a conformational change in the enzyme that helps drive ion transport (Yao and Forte, 2003; Kühlbrandt, 2004; Dunbar and Caplan, 2001; Sachs et al., 1995) (http://www.nlm.nih.gov/cgi/mesh/2011/MB_cgi?mode=&term=Potassium+Hydrogen+ATPase).

The Na^+ - K^+ -ATPase and H^+ - K^+ -ATPase indicate some similarities in the structural building of these enzymes; however, they can be separated from each other by using immunological methods (Stoll and Berglinch, 1987; Sachs, 1987; Chang et al., 1977; Faller et al., 1982; Ganser and Forte, 1973; Lee et al., 1974; 2001; MacLennan et al., 1985; Saccomani et al., 1979 a, b; Sachs, 1977; Sachs et al., 1972; 1980; 1995; Shull et al., 1985; Koenderink et al., 1999; Asano et al., 2003).

5.3. Adenylate cyclase

5.3.1. Short historic background

Sutherland (1951) studied how the hormone epinephrine (adrenaline) signals to regulate the degradation of glycogen to glucose in the liver, thereby increasing glucose output into the blood, so that an organism can respond to stress. He worked alongside future Nobel Prize winners Edmond Fishers and Edwin Krebs, who demonstrated that adenosine triphosphate (ATP) and magnesium were required for phosphorylase activation. Sutherland (1953) agreed to be the chairman of the pharmacology department of Case Western Reserve University in Cleveland and was joined by Theodore R. Rall, "marking the beginning of a long and fruitful period of collaboration between us" [Sutherland EW. Studies on the mechanism of hormone. Science 177: 401–408, 1972 (Lecture delivered 11 December 1971 when he received the Nobel Prize in Physiology or Medicine)]. Sutherland and Rall discovered that epinephrine works by stimulating another chemical messenger, the enzyme glycogen phosphorylase, to begin the sugar-releasing process in the cells (Henion and Sutherland, 1955). This stimulation occurs by means of an intermediary that Sutherland called it the "second messenger," which he identified as a nucleotide and named cyclic AMP (Sutherland et al., 1957; Sutherland and Rall, 1957; Rall and Sutherland, 1957; 1958; Butcher and Sutherland, 1962; 1965; Klainer et al., 1962; Murad et al., 1962). As early as 1960, Sutherland suggested that cyclic AMP acts as a "second messenger" for other hormones as well (Sutherland and Rall, 1958) (for other summaries, see Butcher, 1966; Sutherland, 1962; Sutherland and Rall, 1960; Sutherland et al., 1962; Sutherland and Robison, 1966; András, 1997).

Practically at the same time, the discovery of cyclic 3'-5'-AMP appeared in the organ pharmacology (Sutherland et al., 1965; 1968).

The adenylate cyclase is an enzyme with key regulatory roles in essentially all cells. It is the most polyphyletic known enzyme: six distinct classes have been described, all catalyzing the same reaction, but representing unrelated gene families with no known sequence or structural homology (Class I AC, Class II AC, Class III AC, Class IV AC, Class V AC and Class VI AC).

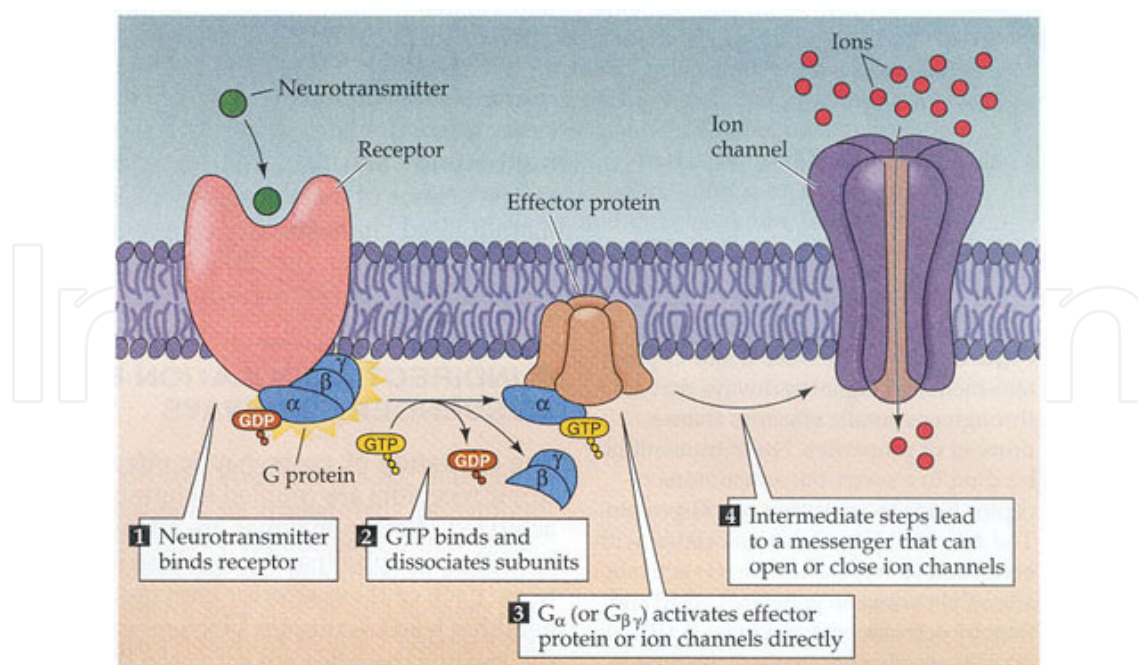


Figure 25. Schematic representation of adenylate cyclase location in the plasma membrane and main steps of intracellular regulatory pathways (<http://www.cs.stedwards.edu/chem/Chemistry/CHem43/>).

The Classes AC I (*E. coli*), II (*Bacillus anthracis* and *Bordetella pertussis*), IV (*Aeromonas hydrophyla*, *Yersinia pestis*), V and VI (*Prevotella ruminicola* and *Rhizobium etli*) are present in different bacteria. The best-known AC class is class III or AC-III (Roman numerals are used for classes). The AC-III occurs widely in eukaryotes and has important roles in many human tissues.

All classes of AC catalyze the conversion of ATP to 3',5'-cyclic adenosine monophosphate (cAMP) and pyrophosphate. Divalent cations (usually Mg^{2+}) are generally present in the enzymatic mechanism. The cAMP produced by AC then serves as regulatory signal via specific cAMP-binding proteins, either transcription factors or other enzymes (e.g., cAMP-dependent kinases).

Most AC-IIIs are integral membrane proteins involved in transducing extracellular signals into intracellular responses. A Nobel Prize in physiology (medicine) was awarded to Earl W. Sutherland (Department of Physiology, Vanderbilt University, Nashville, TN, USA) in 1971 for discovering the key role of AC-III in human liver, where adrenaline directly stimulates AC to mobilize stored energy in the "fight or flight" response (Robinson GA, Butcher RW, Sutherland EW: Cyclic AMP. Academic Press, New York and London, 1971) (two papers published by myself – Mozsik G.: Eur. J. Pharmacol 7: 319, 1969 and in 9: 107, 1970 – were cited by the authors in this book).

The effect of adrenaline is via a G protein-signaling cascade, which transmits chemical signals from outside the cell across the membrane to the inside of the cell (cytoplasm). The outside signal (in this case, adrenaline) binds to a receptor, which transmits a signal to the G protein,

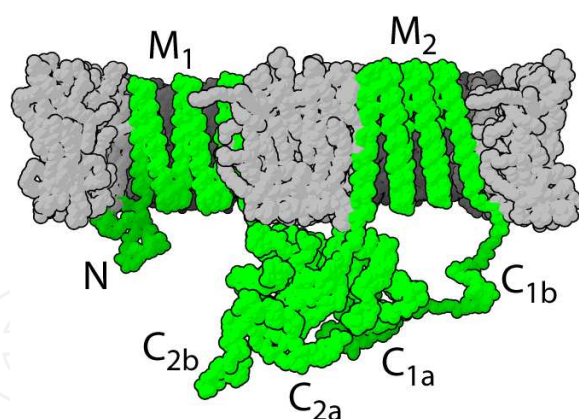


Figure 26. Schematic representation of the structure of adenylate cyclase enzyme (<http://enzyme.expasy.org/EC/4.6.1.1>).

which transmits a signal to adenylate cyclase, which transmits a signal by converting adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). Cyclic AMP is known as the so-called “second messenger” (Reece and Campbell, 2002).

Adenylate cyclases are often activated or inhibited by G proteins, which are coupled to membrane receptors, and thus can respond to hormonal or other stimuli. Following the activation of adenylate cyclase, the resulting cAMP acts as a second messenger by interacting with and regulating other proteins, such as protein kinase A and cyclic nucleotide-gated ion channels.

AC-III structure:

Most class-III adenylate cyclases are transmembrane proteins with 12 transmembrane segments. The protein is organized with six transmembrane segments, the C1 cytoplasmic domain, another six membrane segments and then a second cytoplasmic domain called C1 and C2 regions.

The C1a and C2a sub-domains are homologous and form an intracellular “dimmer” that forms the active site.

There are ten known isoforms of adenylate cyclases in mammals: ADCY1, ADCY2, ADCY3, ADCY4, ADCY5, ADCY6, ADCY7, ADCY8, ADCY9 and ADCY10. These are sometimes called as AC1, AC2, etc., and sometimes Roman numerals are used for these isoforms that all belong to the overall AC class III. They differ mainly in how they are regulated, and are differentially expressed in various tissues throughout mammalian development.

Adenylate cyclase AC-III regulation:

Adenylate cyclase is dually regulated by G proteins (Gs stimulating activity and Gi inhibiting activity) and forskolin, as well as other isoform-specific effectors:

Isoforms III, V and VIII are also stimulated by Ca^{2+} /calmodulin;

Isoforms I and VI are inhibited by Ca^{2+} in a calmodulin-independent manner;

Isoforms II, IV and IX are stimulated by beta–gamma-subunits of the G protein;

Isoforms I, V and VI are most clearly inhibited by Gi, while other isoforms show less dual regulation by the inhibitory G protein.

Soluble AC (sAC) is not a transmembrane form and is not regulated by G proteins or forskolin, instead it acts as a bicarbonate/pH sensor. It is anchored at various locations within the cell and, with phosphodiesterases, forms local cAMP-signaling domain

[(http://www.nlm.nih.gov/cgi/mesh/2011/MB_cgi?mode=&term=Adenylate+cyclase) (http://proteopedia.org/wiki/index.php/Adenylyl_cyclase)].

5.4. Sodium-pump and second messenger system in the gastrointestinal mucosa

5.4.1. Short personal background

After taking short reviews on these biochemical problems in the nature of living cells, we faced a unique situation, when one of us (GyM) received a fellowship possibility in the Department of Pharmacology, University of Blindern, Oslo, in 1968–1969. We encountered these problems in this Department. Furthermore, I worked together with associate professor Ivar Øye, who spent 4 years in the Vanderbilt University (Department of Physiology) and worked together with Professor Sutherland on the problems of cyclic AMP.

After returning home (Norway) from USA, Ivar Øye wanted to do his research in the field of cardiac tissues. He wanted to obtain “plasma membrane” materials, and he wanted to use the “membrane materials” for the study of pharmacological regulation of cyclic AMP transformation in the heart muscles. I was moved to this Department at that time, and I had to receive the critically good evaluation of action of these drugs, all of those were able to inhibit the preparations of “membrane materials” from the heart.

I had some methodological knowledge gained from our earlier biochemical studies of the stomach. However, my new scientific work offered a new challenge in the gastrointestinal mucosal research.

Furthermore, I had some other affinities to enter into this research field, which are as follows:

- a. We earlier mentioned that we were not able to understand and explain the results of clinical pharmacology in patients with peptic ulcer. This is because most of the researchers emphasized the key role of vagus nerve (by the increase of gastric acid secretion); thereby the application of various inhibitory drugs was thought to be reasonable in terms of decrease of gastric acid production. However, if we evaluated critically the actions of these drugs, we had to learn that these drugs are able to inhibit the active metabolism in the gastric tissues. Consequently, we can explain the beneficial effects of these drugs on the healing of peptic ulcer (which was explained by impaired tissue metabolism).

We concluded that we have to do some change in paradigm of the peptic ulcer research. These facts and suggestions led us to study the biochemisms in the gastrointestinal mucosa;

- b.** Many neural, hormonal (and pharmacological) factors, discovered absolutely new mechanisms; later, the immunological events could be collected in participation of peptic ulcer disease. Practically, the researchers studied the influences (action) of one or two factors (especially HCl and pepsin secretion, mucus secretion, bicarbonate secretion, prostaglandins, scavengers and antacids, and their different actions) on the stomach (gastrointestinal tract) in respect to development and protection (healing) of peptic ulcer disease. The results of these observations reflect only indirectly to the gastrointestinal tract.

Besides these results, we were uninformed on the biochemical changes in the gastrointestinal tract in time of gastric acid secretion and mucosal damage versus mucosal protection;

- c.** We wanted to study the biochemism of gastrointestinal tract, and we suggested that the final results (and summary) of different etiological factors can modify the functions of cells in the gastrointestinal tract. The blood supply was a remarkable extracellular factor; however, the changes in the blood flow also produce significant cellular biochemical changes in the target organ;
- d.** A general biochemical approach to study the biochemisms was built up in animal experiments from 1967. Our biochemical methodologies gave absolutely new insight into the biochemism of the gastrointestinal tract.

These biochemical observations were done dominantly on pylorus-ligated rats (as in an acute animal model). One of the most surprising results was to note that similar biochemical changes were detected in both the rumen and glandular parts (Mózsik et al., 1967 a, b). These results led us to study the changes in both the parts of the rat stomach after chronic “surgical vagotomy,” chronic atropine treatment and cholinesterase (neostigmine) inhibitor treatments (for details, refer sections 4.3.1.–4.3.3 of Chapter 4). From the evaluation of the results of clinical pharmacological studies, we conducted biochemical observations in rats after cessation of drug (atropine, cholinesterase inhibitor) treatments.

The evaluation of these biochemical results in rats led us to understand some main lines of cellular biochemistry (cytoplasm, nucleic acids):

- a.** They were reversible in rat gastric tissues;
- b.** They were irreversible also.

The first one accepted the metabolic changes in biochemistry and the second one accepted the metabolic changes in genetic field (nucleic acids). These reversible and irreversible lines in the biochemical changes especially can be seen during the time of chronic “use” and “disuse” of vagus nerve. In some observations, we studied the changes of tissue levels of ATP, ADP (Mózsik et al., 1970a) and base alterations of nucleic acids in the stomach wall after a chronic atropine treatment (Mózsik et al., 1969b).

The results of these observations opened our eyes to start new observations with the main cellular functions of cells;

- c. The international discoveries with membrane-bound ATP-dependent energy systems left a great impression and offered new possibilities to understand the regulations of ATP splitting and its resynthesis in the cells of the gastrointestinal tract;
- d. There was no question that energy liberation exists in all of the functions of membrane-bound ATP-dependent enzymes ($\text{Na}^+\text{-K}^+$ -dependent ATPase, $\text{H}^+\text{-K}^+$ -ATPase, adenylate cyclase). However, the presence of these enzymes was not known in 1968;
- e. In my works (in the Department of Pharmacology of Oslo University, Norway), the biochemical presence of $\text{Na}^+\text{-K}^+$ -ATPase was used in my works to prove clearly that we really work with “membrane preparates,” and these preparates were used to study the functions of adenylate cyclase. It's true that my boss wanted to see and to study these steps in cardiac muscles; however, I used parallelly the gastric mucosa (earlier obtained in rats, later in human gastric resectates) (Mózsik and Øye, 1969), and we wanted to study the actions of different drugs (epinephrine, ouabain, NaF) on the adenylate cyclase. The NaF was used as the most powerful compound to stimulate the adenylase cyclase, because it inhibits the activity of all membrane ATPase activity (this fact is well known in the pertinent literature).

Therefore, consequently, I realized two important things during my study tour in Oslo (Norway), namely to prepare the adenylate cyclase from the crude “membrane materials” (from heart muscles and gastric mucosa) and the presence of $\text{Na}^+\text{-K}^+$ -ATPase was used as a chemical marker for the plasma membrane.

We have to emphasize that the presence of neither $\text{Na}^+\text{-K}^+$ -ATPase nor adenylate cyclase was known in animals and especially in humans;

- f. I suggested that the functions of these enzymes can be modified by different drugs (not by only hormones) under different experimental conditions (including the animal experiments and human medical problems).

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