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Actual Positions in the Research of Membrane-bound ATP-dependent ATP-ase Systems in the World in the Different Tissues and Gastrointestinal Mucosa (in 1968-69)

Gyula Mozsik and Imre Szabó

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During the close of the 1960s, two different workgroups dealt with the problems of active movements of cations across the cell membrane:

- a. Skou (Skou, 1965; Albers, 1967; Askari, 1974; Schwartz et al., 1975; Glynn, 1964) indicated the existence of sodium–potassium pump located in the plasma membrane, which is responsible for the active movements of sodium and potassium. He clearly stated that: (a). this enzyme is located in the plasma membrane; (b) the activity of the enzyme located in plasma membrane can be enhanced by the combined application of sodium and potassium; (c) this enzyme splits the ATP located in the mitochondria into ADP in the presence of magnesium ion and (d) the activity of this enzyme can be specifically inhibited by the application of ouabain (Skou, 1965). The preparation of the membrane enzyme (sodium–potassium-dependent ATPase) was tried to prepare from the amphibian; frog-, rat-, rabbit- gastric mucosa without any success (Kasbekar and Durbin, 1965; Post and Albright, 1961; Sachs et al., 1972).

We used a special treatment for the preparation of plasma membrane and separation or centrifugation, which resulted in a “typical” sodium–potassium-dependent ATPase from the rat and human gastric mucosa (Mózsik and Øye, 1969).

- b. Practically at the same time as the years up to the preparation of sodium–potassium-dependent ATPase (Butcher and Sutherland, 1962; Øye et al., 1964; Øye and Sutherland, 1966; Øye and Butcher, 1967 a, b) discovered another enzyme located in the plasma membrane, which spilled the ATP into 3', 5'-cyclic AMP in the presence of magnesium

Figure 27. First (drugs, hormones) and second messenger systems located in the plasma membrane of cells. [Mózsik et al., *Acta Medica Acad Sci Hung* 36, 1–29, 1979 (with kind permission).]

ions (Butcher and Sutherland, 1962; Øye and Sutherland, 1966; Robinson et al., 1971; Sutherland and Rall, 1957; Butcher, 1966).

We were successful in preparing this enzyme from the human gastric mucosa (Mózsik and Øye, 1969).

It was exciting when we were able to detect the changes in different adenosine phosphates (adenosine triphosphate – ATP, adenosine diphosphate – ADP, adenosine monophosphate – AMP, cyclic 3', 5'-adenosine monophosphate – cAMP) together with the changes in sodium–potassium-dependent ATPase and in adenylate cyclase activities (Figure 27).

We planned to carry out these types of observations under different experimental conditions (with and without drug administration, acute and chronic surgical and chemical vagotomy and with and without application of different necrotizing agents) in rats and patients with different gastric basal acid outputs (BAO) and maximal acid outputs (MAO), and the gastric antral, duodenal and jejunal mucosa around ulcer and the mucosal tissue samples without ulcer.

The energy liberation through the membrane-bound ATP-dependent energy systems (sodium–potassium pump and adenylate cyclase) can be detected well. The ATP–ADP transformation by sodium–potassium–ATPase enzyme releases 7.3 kcal/mol, while the ATP–cAMP transformation by adenylate cyclase results in 2×7.3 kcal/mL. Both the ADP–AMP and cAMP–AMP transformations release 7.3 kcal/mol (Figure 28).

GENERAL BIOCHEMICAL APPROACH TO GI MUCOSA

Figure 28. Pathways of energy liberation from ATP to ADP by membrane ATPase and from ATP to cAMP by adenylylate cyclase located in the plasma membrane. The ATP–ADP transformation releases 7.3 kcal/mol, while ATP–cAMP transformation results in 2×7.3 kcal/mol. On the other hand, ADP–AMP and cAMP–AMP transformations produce 7.3 kcal/mol energy liberation. [Mózsik, Abdel Salam, Király, Morón, Nagy, Sütő, Tárnok, Jávör: in: Mózsik Gy., Nagy L., Király Á. (eds): Twenty Five Years of Peptic Ulcer Research in Hungary: From Basic Science to Clinical Practice. Akadémiai Kiadó, Budapest, pp. 159–170, 1997 (with kind permission).]

DIFFERENT MAIN FACTORS INFLUENCING ON THE ACTUAL TISSUE LEVEL OF ATP

Figure 29. Different regulatory steps in the determination of actual levels of tissues of ATP. The tissue level of ATP is a consequence of ATP breakdown and ATP resynthesis. The limiting factor of ATP resynthesis by the pathway of oxidative phosphorylation is the presence of tissue hypoxia. The presence of tissue hypoxia can be proven biochemically by the increase of lactate levels in the tissue.

6.1. Materials and methods of experiments and human observations

The observations were carried out in CFY (Sprague-Dawley) (Gödöllő, Hungary) strain rats, weighing 180–210 g body weight and on the resectates of stomach and small intestine of patients who underwent gastric surgery because of unhealed ulcer disease (during 1970–1980).

The patients suffered from classical peptic ulcer disease (PUD) together with classical clinical symptoms (decreased appetite, feeling of dullness and pain in epigastric region of the abdomen, pyrosis and impaired gastric emptying and retention syndrome). These patients presented in about one month before the surgical intervention. The presence of gastroduodenal ulcers were endoscopically diagnosed, and thereafter these patients received medical treatments (anticholinergic agents, later H_2 receptor antagonist and antacids for one month time period). The patients who did not heal during this time period were evaluated with the possibility of surgical interventions.

The indication of gastric surgery was given by physicians [with consultations between internists (gastroenterologists vs. surgeons) independently from us] on the resectates of stomach and small intestine (according to the method of Billroth II). A small group of patients underwent classical partial gastrectomy (according to the method of Billroth II) and jejunal ulcer was identified. The patients in this group were also treated medically (pharmacologically) for the same time period as mentioned earlier.

During the time of surgical intervention, the removals of stomach and small intestine were obtained immediately and these were cut into two parts from which one part was given for histological evaluation of resected tissues and the other part was immersed (after separation of mucosa and muscular layers) in liquid nitrogen and used for biochemical examinations. The mucosa specimens were also further separated from each other (depending on the distance of ulcer edge). The biochemical measurements from the mucosa specimens and muscular layers (independently from the number of tissue specimens) obtained from one patient were done (carried out) simultaneously dominantly on the same the as the surgical intervention was carried out.

The animal observations were carried out in both sexes of CFY-strain rats (the used methods are detailed in Section 4.2).

6.2. Membrane-bound ATP-dependent energy systems in the rat and human gastric mucosa

6.2.1. General characterization of membrane ATPase prepared from the rat and human gastric fundic mucosa

The method for preparation of membrane ATPase from the human gastric mucosa was published in 1969 (Mózsik and Øye, 1969; Mózsik et al., 1971 a, b; 1973 a, b; 1974 a, b, c, d; 1975 a, b; 1976 a, b). Figure 30 shows the typical characterization of membrane ATPase from the human gastric fundic mucosa. The membrane ATPase split the ATP into ADP *in vitro* incubation system in the presence of Mg^{2+} (Mg^{2+} -dependent), which can be enhanced by the combined application of Na^+ and K^+ (Mg^{2+} - Na^+ - K^+ -dependent ATPase), in liberation of inorganic phosphorus followed by the ATP splitting process. The total ATPase can be inhibited by the application of ouabain (g-strophanthin). The difference between the total (Mg^{2+} - Na^+ - K^+

$^{+}$ -dependent) versus only Mg^{2+} -dependent ATPase is given as Na^{+} - K^{+} -dependent ATPase (Mózsik et al., 1978 a, b, c). This Na^{+} - K^{+} -dependent ATPase has been associated with the active movements of Na^{+} and K^{+} (Skou, 1967; Albers, 1967; Askari, 1974; Glynn, 1964).

Figure 30. Biochemical characterization of membrane ATPase prepared from human gastric fundic mucosa. The membrane ATPase activity was measured by the liberation of inorganic phosphate from ATP produced by membrane ATPase *in vitro* incubation system in the presence of Mg^{2+} , Mg^{2+} , Na^{+} and K^{+} (total ATPase). The difference between the total and only magnesium-dependent ATPase activity is equal to Na^{+} - K^{+} -dependent ATPase activity. The g-strophanthin (ouabain) is able to inhibit the Na^{+} - K^{+} -dependent ATPase (means $\pm$ SEM). [Mózsik et al.: Acta Physiol Scand Spec Suppl. 199–208; 1978 (with kind permission).]

There was a question whether there is any correlation between the neural regulation Andana $^{+}$ - K^{+} -dependent ATPase. A log-concentration dose–response curve for acetylcholine, prepared from the human gastric fundic mucosa, was identified (Csáky, 1969; Mózsik et al., 1978 a, b, c) (Figure 31).

6.2.2. Determination of affinity and intrinsic activity curves for the dugs modifying the Na^{+} - K^{+} -dependent ATPase activity obtained from rat and human gastrointestinal mucosa

The affinity and intrinsic activity curves were determined and calculated according to the method of Csáky (Csáky, 1969). The intrinsic activity (α) of ouabain was taken to be equal to 1.00. The values of pD_2 (dose necessary to produce 50% inhibition in affinity curves) and pA_2 (the dose necessary to produce 50% inhibition in the intrinsic curves) were calculated from affinity and intrinsic activity curves.

Figure 31. Log-concentration curve of acetylcholine (ACh) on the membrane ATPase prepared from the human gastric fundic mucosa. [Mózsik et al.: Acta Physiol Scand Suppl 199–208; 1978 (with kind permission).]

Figure 32. Dose-response curves for the drugs on the sodium-potassium-dependent ATPase prepared from human gastric, duodenal and jejunal mucosa (affinity curves). [Mózsik, Kutas, Nagy, Tárnok: Acta Medica Acad Sci Hung 36:459–466; 1979c (with kind permission).]

The dose-response curves (affinity curves) of the drugs were analyzed on the Na⁺-K⁺-dependent ATPase prepared from the human gastric fundic, duodenal and jejunal mucosa (Mózsik et al., 1979 a, b, c, d, e) (Figures 32–34).

Figure 33. Dose-response curves for drugs inhibiting the $\text{Na}^+\text{-K}^+$ -dependent ATPase prepared from the human gastric fundic mucosa. The intrinsic activity (α) of ouabain to $\text{Na}^+\text{-K}^+$ -dependent ATPase system was taken to be equal to 1.00 and the effects of different drugs were compared with its effect; n indicates the number of patients. The values of intrinsic activities of drugs to $\text{Na}^+\text{-K}^+$ -dependent ATPase system were calculated from the curves and presented in Table 3. [Mózsik, Kutas, Nagy, Tárnok: Acta Medica Acad Sci Hung 36:459-466, 1979c (with kind permission).]

AFFINITY AND INTRINSIC ACTIVITY CURVES FOR EPINEPHRINE cAMP, AMP DUE TO THE $\text{Na}^+\text{-K}^+$ -DEPENDENT ATP-ASE IN THE GASTRIC MUCOSA OF RATS

Figure 34. Affinity and intrinsic activity curves of epinephrine, cAMP and AMP on $\text{Na}^+\text{-K}^+$ -dependent ATPase prepared from the rat gastric fundic mucosa. [Mózsik Gy, Garamszegi M, Jávör T, Nagy L, Patty I, Sütő G, Vincze Á: Ann N Y Acad Sci 597:264-281, 1990a (with kind permission).]

Drugs	Affinities (pD ₂)	Intrinsic activities	
		(α)**	(pA ₂)
Atropine	9.50	1.00	9.50
Epinephrine	8.60	0.41	8.60
cAMP	11.30	0.48	11.30
PGE ₁	9.30	1.00	8.30
PGE ₂	9.45	1.00	9.45
Pentagastrin	10.55	0.87	10.55
Histamine	9.70	1.00	9.70
Ouabain	7.50	1.00	7.50

Table 26. Characterization of the actions of drugs inhibiting Na⁺-K⁺-dependent ATPases prepared from human fundic mucosa.* expressed in [-], ** $\alpha_{\text{ouabain}}=1.00$

The values of pD₂ and pA₂ were calculated from the measured affinity and calculated intrinsic activity curves (Table 26).

6.3. Adenylate cyclase preparation from the rat and human gastric fundic mucosa

The adenylate cyclase was originally and first prepared from the human gastric fundic mucosa (Mózsik, 1969b; Mózsik et al., 1970b). Similar observations and results were done and obtained from the rat fundic mucosa (Ruoff and Sewing, 1974; Salganik and Bersimbaev, 1976; Thompson et al., 1977 a, b).

The fresh gastric mucosal tissues of rats and humans were put into 20 mL of ice-cold 0.25 M sucrose solution, and it was homogenized in the room temperature of 4°C, then centrifugation was done at 0°C with 2000 g for 20 minutes. The supernatant material was removed and the sediment was resuspended in 25 mL ice-cold glycyl-glycin buffer solution (264 mg of glycyl-glycin and 24.65 mg of MgSO₄ were immersed in 100 mL of distilled water, and its pH value was fixed at 7.8). The rehomogenization was done, and thereafter centrifugation was carried out with 2000 g value for 20 minutes at 0°C. The supernatant material was removed after centrifugation, and the sediment was resuspended in 20 mL glycyl-glycin buffer solution. Two-milliliter samples from this sediment solution were put in other tubes and centrifuged with 2000 g for 10 minutes at 0°C. After this centrifugation, the supernatant material was removed and the sediment was resuspended in 150- μ L glycyl-glycin buffer solution and it was used for measurements of adenylate cyclase.

The incubation solution (150 μ L of resuspended enzyme, 50 μ L of tested compounds: H₂O, epinephrine, NaF, atropine, acetylcholine, 150 μ L Tris-buffer, 50 μ L C¹⁴-ATP – 20–25 mC/mmol dissolved in Mg solution) was used for 10 minutes at 30°C temperature before the measurements of enzyme activity of adenylate cyclase. The enzyme activity was stopped after boiling for 3 minutes; however, before the heat treatment 20 μ L H³-cyclic 3', 5'-adenosine monophosphate was added into incubated samples.

The ATP, ADP, cyclic AMP and AMP were separated from the 300 μL of incubated suspension by Dowex 50 ion exchange column (Krishna et al., 1968), and three peaks were obtained. The first peak contained the ATP and ADP, the second one the cyclic AMP and the third one the 5'-AMP. The other components (such as nucleotides, inorganic phosphate and cyclic 3', 5'-AMP) were then precipitated by the treatment of $\text{ZnSO}_4\text{-Ba}(\text{OH})_2$. After centrifugation of these samples, the supernatants were evaporated and thereafter the residue was immersed in 10 mL of Bay's solution. The radioactivity of cyclic AMP was detected by liquid scintillation equipment (H. Pacard) of H^3 and C^{14} labelled cyclic 3', 5'-AMP. The extent of the ATP—AMP transformation was given as counts of C^{14} counts of 100 H^3 (see Table 7) .

Examined compounds	Incubation time (min)	C^{14} / 100 H^3
1. H_2O	0	12.36
2. H_2O	10 (control)	23.96
2. Epinephrine (10^{-4} M)	10	28.43
4. NaF (10^{-2} M)	10	35.84
5. Atropine (10^{-4} M)	10	29.60
6. Acetylcholine (10^{-4} M)	10	21.52

Table 27. Testing of the effects of epinephrine, NaF, atropine and acetylcholine on the transformation of ATP into cyclic AMP by adenylate cyclase prepared from the human gastric mucosa (Mózsik, 1969a: PhD Dissertation of Pécs University, Hungary)

6.4. Feedback mechanism systems between the membrane-bound ATP-dependent energy systems in the gastrointestinal mucosa in rats and humans

6.4.1. Pharmacological regulatory mechanisms between the membrane ATPase (ATP – ADP transformation) and adenylate cyclase (ATP – cAMP transformation) prepared from the rat and human gastric fundic mucosa

The origin of the suggestion to existence of feedback system between the $\text{Na}^+\text{-K}^+$ -dependent ATP and adenylate cyclase was created by me (GyM) based on the results of personal observations and after studying the actions of different drugs on these enzymes.

It was interesting to compare the effects of drugs on $\text{Na}^+\text{-K}^+$ -dependent ATPase and adenylate cyclase activity from different “membrane fractions” under *in vitro* conditions (Table 28). The $\text{Na}^+\text{-K}^+$ -dependent ATPase activity was inhibited by epinephrine, NaF and atropine (Mózsik,

Na ⁺ -K ⁺ -dependent ATPase activity			
Drugs	Effects	Tissue specimens	
Adrenaline	Inhibition (10 ⁻¹⁰ to 10 ⁻⁴ M)	rat heart rat gastric mucosa human gastric mucosa	
NaF	Inhibition (10 ⁻³ to 10 ⁻² M)	rat heart rat gastric mucosa human gastric mucosa	
Atropine	Inhibiton (10 ⁻¹⁰ to 10 ⁻⁴ M)	rat heart rat gastric mucosa human gastric mucosa	
Acetylcholine	No effect (10 ⁻¹⁰ to 10 ⁻⁴ M)	rat heart rat gastric mucosa human gastric mucosa	
Adenylate cyclase activity			
	Effecs	Tissue specimens	References to adenylate Cyclase
Adrenaline	Stimulation (10 ⁻⁷ to 10 ⁻⁴ M)	rat heart rat gastric mucosa human gastric mucosa	Murad et al., 1962 Mózsik, 1969a,b
NaF	Stimulation (10 ⁻³ to 10 ⁻² M)	rat heart rat gastric mucosa human gastric mucosa erythrocyte	Sutherland et al., 1962 Mózsik, 1969a, b Øye and Sutherland, 1966
Atropine	Stimulation (10 ⁻⁵ to 10 ⁻⁴ M)	rat heart rat gastric mucosa human gastric mucosa	Murad et al., 1962a, b Mózsik, 1969
Acetylcholine	Inhibition (10 ⁻⁵ to 10 ⁻⁴ M) (30 %)	rat heart	Murad et al., 1962

Table 28. Effects of drugs used on Na⁺-K⁺-dependent ATPase and adenylate cyclase activity by different “membrane preparates” prepared from rat heart, rat and human gastric mucosa under *in vitro* conditions (Mózsik, 1969 a, b).

1969b), whereas the adenylate cyclase activity was stimulated by adrenaline (epinephrine) (Murad et al., 1962; Klainer et al., 1962; Øye and Sutherland, 1966), NaF (Sutherland, et al., 1962; Øye and Sutherland, 1966) and by tropine (5 to 20 % applied in 10^{-5} and 10^{-4} concentrations) (Murad, Chal., 1962). The adenylate cyclase activity was inhibited by acetylcholine and acetyl- β -methylcholine (Murad, Chi, R 1962). Acetylcholine had no effect on Na^+ – K^+ -dependent ATPase activity (consequently, the ATP transformation into ADP is working without any trouble during choline's effect on Na^+ – K^+ -dependent ATPase).

When adenylate cyclase activity is studied in course particle preparations having high ATPase activity, the local concentration of ATP at the catalytic site of adenylate cyclase might be rate limiting for the reaction. An inhibition of Na^+ – K^+ -dependent ATPase by adrenaline, NaF and atropine might therefore apparently stimulate the adenylate cyclase activity (by the increase of ATP concentration). It was particularly interesting that NaF not only inhibited Na^+ – K^+ -ATPase activity, but also stimulated the adenylate cyclase activity more than other drugs. The inhibition of adenylate cyclase activity by acetylcholine is due to a decrease in local concentration of ATP caused by Na^+ – K^+ -dependent ATPase activity at the catalytic site of adenylate cyclase. The inhibitory effect of acetylcholine on adenylate cyclase activity was prevented by adding atropine in a concentration of 10^{-7} M leading to an inhibitory effect on Na^+ – K^+ -dependent ATPase; there was no effect of this concentration of atropine alone on the adenylate cyclase (Murad et al., 1962).

The inhibition caused by adrenaline and atropine was present in concentrations from 10^{-9} to 10^{-4} M (Mózsik, 1969 a, b), whereas the stimulation by adrenaline and atropine on adenylate cyclase activity was obtained by concentrations from 10^{-6} to 10^{-5} M (Klainer et al., 1962; Murad et al., 1962; Mózsik, 1969 a, b). Thus, *in vitro* Na^+ – K^+ -dependent (active transport) ATPase system was 100–1000 times more sensitive to drugs than the adenylate cyclase system.

The difference in sensitivity between the Na^+ – K^+ -dependent (transport) ATPase and adenylate cyclase activity, and the contradictory effects of drugs on them indicated the following:

1. The stimulatory effects by these drugs on adenylate cyclase activity is associated with blocking of Na^+ – K^+ -dependent ATPase activity;
2. The Na^+ – K^+ -dependent ATPase (transport) system and adenylate cyclase system can be separated.

A direct inhibitory effect of cyclic AMP and adenosine 5'-monophosphate (5'-AMP) of Na^+ – K^+ -dependent ATPase activity from human gastric mucosa was observed in a wide range of concentrations (Mózsik, 1970). The cyclic 3', 5'-AMP and 5'-AMP are present in the cells as products of adenylate cyclase activity. The direct inhibition of Na^+ – K^+ -dependent (active transport) ATPase by cyclic 3', 5'-AMP and 5'-AMP showed an antagonistic interrelationship between the adenylate cyclase system and the active transport ATPase system (Figure 35).

In the forthcoming years and thereafter, the tissue levels of ATP, ADP, cyclic AMP, AMP (adenine–adenosine) were measured *in vivo* conditions (in different human gastrointestinal diseases and under different animal experiments) directly from the gastrointestinal mucosal

Figure 35. The summary of some feedback mechanisms of drugs with interrelationship between the active transport ($\text{Na}^+\text{-K}^+$ -dependent ATPase) system and the adenylate cyclase prepared from rat and human gastric mucosa (Mózsik, 1969b). These types of observations were carried out under *in vitro* conditions (with kind permission).

tissues. However, the details of the above entioned hypothesis iere taken into account for the evaluation of the forthcoming observations.

These pharmacological regulatory functions were opposite ones on the $\text{Na}^+\text{-K}^+$ -dependent ATPase and adenylate cyclase (Tables 28, 29). Generally, different drugs were able to modify the $\text{Na}^+\text{-K}^+$ -dependent ATPase in lower molar concentrations than those in the adenylate cyclase concentrations. These results a priori suggested the existence of a complex feedback system, which could be modified by mediators, hormones and drugs.

6.4.2. Regulatory mechanisms between the tissues levels of ATP, ADP, cAMP and AMP in the intact rat gastric mucosa (in vivo studies)

The tissue levels of ATP, ADP and AMP were enzymatically (Boehringer Ingelheim, Germany); while the cAMP by RIA (Beckton, Dickinson, Orengeburg, USA) was measured. The protein content was measured by the method of Lowry et al. (1951) in the tissue concentration of ATP, ADP and AMP (except for cAMP which was expressed as pmol/mg) in nmol/mg protein. The ratio of ATP/ADP and the values of adenylate pool (ATP+ADP+AMP) were calculated. From the ATP, ADP, AMP and adenylate pool, the values of "energy charge" $[(\text{ATP} + 0.5 \text{ ADP}) / (\text{ATP} + \text{ADP} + \text{AMP})]$ were calculated according to the method of Atkinson's formula (1968). This value is theoretically 1 when all adenosine compounds are in phosphorylated form, and its value is 0 when all adenosine nucleotides are in dephosphorylated form.

Drugs	ATP-ADP transformation			ATP-cAMP transformation		
	Effects	Doses (M)		Effects	Doses (M)	
Acetylcholine	Stimulation	10^{-7} M	→	Inhibition	10^{-4} M	→
Parasympatholytics:						
Atropine	Inhibition	10^{-11} M	→	Stimulation	10^{-8} M	→
Isopropamide	Inhibition	10^{-8} M	→	Stimulation	10^{-5} M	→
Gastrixon	Inhibition	10^{-7} M	→	Stimulation	10^{-4} M	→
Epinephrine	Inhibition	10^{-9} M	→	Stimulation	10^{-7} M	→
β-blocker (Visken)	Stimulation	10^{-4} M	→	Inhibition	10^{-5} M	→
Histamine	Inhibition	10^{-11} M	→	Stimulation	10^{-8} M	→
Cimetidine	Stimulation(?)	10^{-4} M	→	Inhibition	10^{-6} M	→
Pentagastrin	Inhibition	10^{-11} M	→	Stimulation	10^{-9} M	→
PGE ₁ Inhibition	10^{-11} M	→	Stimulation	10^{-9} M	→	
PGE ₂ Inhibition	10^{-11} M	→	Stimulation	10^{-9} M	→	
Ouabain	Inhibition	10^{-8} M	→	Stimulation	10^{-4} M	→
cAMP Inhibition	10^{-13} M	→				
AMP Inhibition	10^{-8} M	→				

*Direct effects of the membrane-bound ATP-splitting enzyme activities.

Table 29. Pharmacological effects on the transformation of ATP into ADP by membrane ATPase and the ATP-cAMP transformation by adenylate cyclase from rat and human gastric, fundic, antral, duodenal and jejunal mucosa. [Mózsik and Jávör: Dig. Dis. Sci 33:92–105, 1988* (with kind permission).]

Some general aspects of the biochemical approach need to be emphasized for the establishment of the theoretical base of the feedback systems between the tissue levels of ATP, ADP, AMP and cAMP:

1. Simultaneous determinations of the tissue ATP, ADP, AMP and cAMP and lactate may be used for a correct evaluation of the role of membrane-bound ATP-dependent energy systems in both development and prevention of ulcer. No such conclusions can be derived from the determination of ATP or cAMP alone;
2. The tissue hypoxia produced by two characteristic biochemical properties: (a) a significant increase in the level of lactate in tissue and (b) failure of the ATP resynthesis due to impaired oxidative phosphorylation by tissue hypoxia. A significant decrease in tissue ATP level was observed in many experimental models, but without an increase in the lactate level (Mózsik et al., 1976 a, b, c, d; Mózsik and Vizi, 1976 a, b; Mózsik et al., 1979 a, b, c, d, e, f, g; Mózsik et al., 1981 a, b, c, d, e; Nagy et al., 1983). The simultaneous measurements of ATP-membrane, ATPase-ADP and ATP-adenylate cyclase-cAMP systems offer useful information on the energy turnover. If there was increased activity of ATP-splitting enzymes together with increased concentration of ADP and cAMP, it would be indicative of increased ATP breakdown in one or another direction, or in both pathways (Mózsik and Vizi, 1976 a, b; Mózsik et al., 1978c; Mózsik et al., a, b, c, d, e, f, g; Mózsik et

al., 1983 a, b, c). If the ATP level had been found at a significantly higher level, this would indicate an increased extent of oxidative phosphorylation. These findings however do not indicate the presence of tissue hypoxia in the gastrointestinal mucosa;

3. The balance between the membrane-bound ATP-dependent energy systems can be modified by hormones, mediators and drugs, under physiological and pathophysiological conditions (Mózsik et al., 1979 a, b, c, d, e, f, g; Mózsik et al., 1983 a, b, c). The major effects produced by these agents can be evaluated as steps in the injury to the gastrointestinal mucosa. It is important to note that the ATP-ADP and ATP-cAMP energy systems are affected by different dose-response curves of drugs. For example, the doses required to produce 50% inhibition of ATP-ADP and ATP-cAMP transformation from affinity curves (pD_2) and those required to express intrinsic activities (pA_2) using ouabain as having an activity ($\alpha = 1.00$) differ significantly in both systems (Tables 26, 29);
4. Data are scarce for the two ATP-dependent energy systems (Robinson et al., 1957; Schwartz et al., 1975; Sutherland and Rall, 1975, 1960; Butcher and Sutherland, 1971). Their correlations were evaluated by responses to various hormones, mediators and drugs by the gastric mucosa in rats and human subjects. These responses show that feedback mechanisms exist between the membrane-bound energy systems (Mózsik et al., 1979 a, b, d, e, f, g; Mózsik, 1969 a, b);
5. The system is characterized mainly by the following:
 - a. ATP is a common substrate for both Na^+K^+ -dependent membrane ATPase and adenylate cyclase (Mózsik, 1969 a, b);
 - b. the drugs which stimulate membrane ATPase activity, inhibit the transformation of ATP into cAMP by adenylate cyclase (Mózsik, 1969 a, b);
 - c. drugs and hormones inhibiting the membrane ATPase activity stimulate the adenylate cyclase activity directly and vice versa (Mózsik et al., 1973 a, b; Mózsik et al., 1974 a, b, c; Mózsik et al., 1979 a, b, c, d, e, f; Mózsik et al., 1981 a, b, c);
 - d. the increased transformation of ATP into ADP indirectly inhibits the extent of ATP-cAMP transformation (Mózsik et al., 1983b; Morón et al., 1983; Mózsik et al., 1983 b, c);
 - e. the decrease in transformation of ATP into ADP by various agents leads to the indirect stimulation of the transformation of ATP into cAMP (Mózsik et al., 1979 a);
 - f. the increase in ATP transformation into cAMP leads to an indirect inhibition of ATP-ADP transformation (Mózsik et al., 1979 a, b, c, d, e, f, g, h);
 - g. AMP and cAMP directly inhibit the transformation of ATP into AMP by direct inhibition of membrane ATPase activity (Mózsik, 1969a, 1970);
 - h. the inhibition in the transformation of ATP into cAMP is the result of the indirect stimulation in the transformation of ATP into ADP (Mózsik et al., 1987 a, b);

- i. the extent of cAMP transformation through regulation of phosphodiesterase by drugs may be regulated by both ATP–ADP and ATP–cAMP transformation.
6. The extent of phosphorylation and/or dephosphorylation can be estimated by the Atkinson's formula (1968).

The critical evaluation of the results of these observations offers a significant possibility for the biochemical background of gastrointestinal tract.

Figure 36. Main regulatory steps to membrane-bound ATP-dependent energy systems in rats under the physiological conditions. [Mózsik et al., Ann N Y Acad Sci. 597: 264–281, 1990a (with kind permission).]

6.4.3. Cholinergic and adrenergic influences on the regulatory mechanisms between the tissue levels of ATP, ADP, cAMP and AMP in the intact rat stomach

Under the physiological conditions, the ATP–ADP transformation is the first-line regulation in the active movements of cations (Na⁺, K⁺) across the cell membrane, and this ATP decrease in the membrane ATPase results in an indirect inhibition on the ATP–cAMP transformation. Furthermore, the acetylcholine (ACh) alone directly inhibits the adenylate cyclase activity (Mózsik, 1969 a, b). The decrease in cellular level of cAMP relatively enhances the extent of the ATP–ADP transformation, and in the meantime further decreases the extent of ATP–cAMP transformation (Figure 38).

Epinephrine directly enhances the adenylate cyclase activity and it directly inhibits the membrane ATPase activity in smaller doses (Mózsik, 1970). During the epinephrine effect, the extent of ATP–cAMP transformation will increase, and in the meanwhile the ATP–ADP transformation will decrease (Figures 16, 17).

Figure 37. Pharmacological regulations (first messengers) of membrane-bound ATP-dependent energy systems (second messengers) in the rat and human gastric mucosa under the physiological conditions. [Mózsik et al., Acta Medica Acad Sci Hung 36, 1-29, 1979 (with kind permission).]

REGULATORY MECHANISMS BETWEEN MEMBRANE-BOUND ATP-DEPENDENT CELLULAR ENERGY SYSTEMS UNDER SYMPATHETIC NERVE EFFECT

Direct effects:

1. inhibition of Na⁺-K⁺-dependent ATPase by epinephrine (from 10⁻⁹ M), cAMP (from 10⁻¹¹ M) and AMP (from 10⁻⁸ M)
2. stimulation of adenylate cyclase by epinephrine (from 10⁻⁵ M)

Indirect effects:

increased or decreased tissue levels of substrates (ATP, cAMP, ADP, AMP) modify the final results of their splitting enzymes.

Figure 38. Epinephrine-induced direct and indirect regulatory mechanisms on the transformation of ATP-ADP by membrane ATPase and of ATP-cAMP by adenylate cyclase and of ADP-AMP and cAMP-AMP transformation in the gastric mucosa in intact rats. [Mózsik et al., Ann N Y Acad Sci 579, 264-281, 1990a (with kind permission).]

REGULATORY MECHANISMS BETWEEN THE GASTRIC MUCOSAL MEMBRANE-BOUND ENERGY SYSTEMS UNDER NORMAL (INTACT), ADRENERGIC AND CHOLINERGIC NEURAL EFFECTS

Figure 39. Regulatory mechanisms between the cellular ATP, ADP, cAMP and AMP in the rat gastric mucosa under normal (intact), adrenergic and cholinergic neural effects.

6.4.4. The effects of “surgical” and “chemical” vagotomy on the energy metabolism of gastric mucosa in intact rats

The aims of these studies were as follows:

- To evaluate the effects of acute “chemical” and “surgical” vagotomy on the gastric mucosal biochemistry in rats;
- To find correlations between these tissue biochemical parameters after “surgical” and “chemical” vagotomy (without the application of any damaging agents) in intact rats.

Figure 40. Experimental design of these experiments. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol S135–S139, 1992b (with kind permission).]

Figure 41. Surgical and chemical vagotomy-induced changes in the gastric mucosal levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in rats (without giving any necrotizing agents). *P* values are between the intact (sham-operated) versus surgical and chemical vagotomized rats. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2):S135–S139, 1992b (with kind permission).]

Figure 42. "Surgical" and "chemical" vagotomy-induced changes in the tissue levels of cyclic adenosine monophosphate (cAMP), ratio of ATP/ADP and "energy charge" in the gastric fundic mucosa of rats (without application of any necrotizing agents). For further explanations, see Figure 41. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2): S135–S139, 1992b (with kind permission).]

Figure 43. “Surgical” and “chemical” vagotomy-induced changes in the gastric mucosal levels of the adenylate pool (ATP+ADP+AMP) and lactate. For further explanation, see Figure 41. [Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2): S135–S139, 1992b (with kind permission).]

	Transformations of		
	ATP – ADP	ATP – c AMP	c AMP – AMP
Intact vagus	↑↑↑	↓↓↓	↓↓↓
Surgical vagotomy	0 ← P<0.05	0 ← P<0.001	0 ← P<0.001
Pharmacological vagotomy	↓↓	↑↑↑	↓↓↓

Figure 44. Surgical” and “chemical” vagotomy-induced changes in the gastric mucosal membrane-bound ATP-dependent energy systems of rats (without application of any necrotizing agents). *P* values are presented between intact (sham-operated) versus “surgical” and “chemical” vagotomized groups: ↑, increase; ↓, decrease. Abbreviations: 0, not significant; ↓↓, *P* < 0.01; ↑↑↑ (↓↓↓): *P* < 0.001. (Mózsik, Sütő, Vincze: J. Clin. Gastroenterol 14(Suppl.2): S135–S139, 1992b) (with kind permission).

The results in this chapter clearly indicate that the acute “surgical” and “chemical” (acute administration of atropine) vagotomy produce significantly different changes in the gastric mucosal energy systems (similar conclusions were obtained from our biochemical observations carried out earlier).

Membrane-bound ATP-dependent (ATP-splitting) systems			
Mg ²⁺ -Na ⁺ -K ⁺ -dependent ATPase			
Compounds	Mg ²⁺ -dependent	Na ⁺ -K ⁺ -dependent	Adenylate cyclase
Acetylcholine	No effect	Stimulation	Inhibition
Atropine	No effect	Inhibition	Stimulation
cAMP	No effect	Inhibition	No effect
AMP	Stimulation	Inhibition	No effect

Table 30. Effect of compounds on membrane- bound ATP-dependent systems. [Mózsik, Jávör: Dig. Dis. Sci. 33:92–105, 1988; Mózsik, Nagy, Tárnok, Vizi: Acta Med. Acad. Sci. Hung. 36: 1–29, 1979) (with kind permission).]

Author details

Gyula Mozsik^{1*} and Imre Szabó²

*Address all correspondence to: gyula.mozsik@aok.pte.hu

1 First Department of Medicine, Medical and Health Centre, University of Pécs, Hungary

2 Associate Professor at the First Department of Medicine, University of Pécs, Hungary