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Effect of Activators and Blockers of Ligand-Regulated Ion Channels on the Activity of the Cl^--Stimulated Mg^{2+}-ATPase of the Plasma Membrane Fraction from Bream (*Abramis brama* L.) Brain

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Abstract—Effects of GABA, glycine, acetylcholine, and glutamate (agonists of the $GABA_a$/benzodiazepine, glycine, choline, and glutamate receptors, respectively) at concentrations in the range 10^{-8}-10^{-4} M on the activity of "basal" Mg^{2+}-ATPase of the plasma membrane fraction from bream brain and on its activation by Cl^- were investigated. GABA and glycine activated "basal" Mg^{2+}-ATPase activity and suppressed its activation by Cl^-. Acetylcholine and glutamate activated "basal" Mg^{2+}-ATPase to a lesser extent and did not suppress the activation of the enzyme by Cl^-. The activation of "basal" Mg^{2+}-ATPase by neuromediators was decreased by blockers of the corresponding receptors (picrotoxin, strychnine, benztropine mesylate, and D-2-amino-5-phosphonovaleric acid). In addition, picrotoxin and strychnine eliminated the inhibiting effect of GABA and glycine, respectively, on the Cl^--stimulated Mg^{2+}-ATPase activity. Agonists of the $GABA_a$/benzodiazepine receptor—phenazepam (10^{-8}-10^{-4} M) and pentobarbital (10^{-6}-10^{-3} M)—activated the "basal" Mg^{2+}-ATPase activity and decreased the Cl^--stimulated Mg^{2+}-ATPase activity. The dependence of both enzyme activities on ligand concentration is bell-shaped. Moreover, phenazepam and pentobarbital increased the "basal" Mg^{2+}-ATPase activity in the presence of 10^{-7} M GABA and did not influence it in the presence of 10^{-4} M GABA and 10^{-6} M glycine. The data suggest that in the fish brain membranes the Cl^--stimulated Mg^{2+}-ATPase interacts with $GABA_a$/benzodiazepine and glycine receptors but not with *m*-choline and glutamate receptors.

Key words: fish, brain, plasma membranes, Mg^{2+}-ATPase, chloride, GABA, glycine, acetylcholine, glutamate, picrotoxin, strychnine, benztropine mesylate, pentobarbital, phenazepam, D-2-amino-5-phosphonovaleric acid

In 1965, Durbin and Kasbekar were the first to report anion-stimulated Mg^{2+}-ATPase in frog gastric mucosa, and they suggested that it participates in ATP-dependent Cl^-/HCO_3^--exchange across the cell plasma membrane [1]. Since then the properties of the enzyme have been studied in the cell membranes of animals of different levels of organization [2]. The activity of the anion-stimulated Mg^{2+}-ATPase (P-type ATPase, EC 3.6.1.3) is determined as anion-stimulated (especially HCO_3^- and/or Cl^-) ATP hydrolysis revealed in the presence of ATP and Mg^{2+}, whose optimal ratio varies depending on tissue [2-4]. A series of studies have shown that high concentrations of the substrate and Mg^{2+} and also high molarity of the buffer mask the stimulating effect of anions [2, 5, 6]. The anion-stimulated Mg^{2+}-ATPase activity of the plasma membranes from different cells is inhibited by blockers of Cl^--transport (furosemide, ethacrynic acid), inhibitors of anion transport (stilbene derivatives SITS and DIDS, thiocyanate), reagents for SH-groups (*p*-chloromercuribenzoate, N-ethylmaleimide), an inhibitor of carboanhydrase (acetazolamide), and a blocker of H^+-ATPase and Na^+,K^+-ATPase (oligomycin) [2-8]. The blockers of anion-stimulated Mg^{2+}-ATPase also inhibit so-called basal Mg^{2+}-ATPase activity, i.e., Mg^{2+}-ATPase activity that is revealed in the absence of anions [5, 7]. It was established that the fraction of the "basal" Mg^{2+}-ATPase inhibited by blockers of anion transport is also related to the anion-stimulated Mg^{2+}-ATPase; therefore, its activity and kinetic properties are studied along with the properties of the anion-stimulated Mg^{2+}-ATPase [3, 5, 7].

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The HCO_3^--stimulated Mg^{2+}-ATPase has been studied in cell membranes of different origin and its properties are thoroughly described (for review, see [2, 3]). The Cl^--stimulated Mg^{2+}-ATPase is studied much less. This enzyme was found in avian salt glands [6], in rat brain [7], in gills and olfactory mucosa of fish [5], in gut of mollusk [9] and rabbit [8], and in a number of other tissues [4, 9]. The Cl^--stimulated Mg^{2+}-ATPase from neuronal cells of animals has been most extensively studied. Thus, in the plasma membranes of rat brain, ethacrynic-inhibited, anion-sensitive Mg^{2+}-ATPase (Cl^--ATPase) was found [7]. The enzyme was purified, its subunit composition was determined, and its kinetic properties were studied [10]. In addition, it was shown that the Cl^--ATPase from the plasma membranes of rat brain [11, 12] and of fish olfactory mucosa [5] and ATP-dependent $^{36}Cl^-$ transport across vesicular membranes (including artificial liposomes [11]) are similar in their responses to the blockers (ethacrynic acid, furosemide, SITS) and pH and in their affinities to substrate (K_m) and chloride ions. These data suggested that the brain Cl^--ATPase from animals is an ATP-dependent Cl^--pump translocating Cl^- from inside the cell to the extracellular space [12], where its concentration is higher than in cytoplasm [13]. However, direct proofs of the participation of the Cl^--ATPase in ion transport against the electrochemical gradient, similar to that shown for Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase, are not yet available [14].

It is known that acetylcholine, serotonin, and noradrenaline, via their corresponding receptors, regulate the functioning of the Na^+,K^+-ATPase from mammalian brain [15], converting the enzyme from dimeric to monomeric form [16] and thus decreasing its activity. In connection with this, of particular interest are data on the influence on the Cl^--ATPase of ligands that control Cl^--channels via $GABA_a$ and glycine receptors. It was shown that the transduction of a potential in fish olfactory receptors is inhibited by $GABA_a$-ergic compounds [17], and the Cl^--ATPase activity of the neuronal membranes of fish olfactory mucosa, which supposedly participates in the potential generation [18], is inhibited by the ligands of glycine and $GABA_a$ receptors [19]. Thus it became clear that the Cl^--ATPase activity, similarly to the Na^+,K^+-ATPase, is decreased by ligands regulating the receptor proteins. Furthermore, it was shown that the Cl^--ATPase from the plasma membranes of fish brain is also affected by the ligands of $GABA_a$ receptors. In particular, γ-aminobutyric acid (GABA) stimulates the "basal" Mg^{2+}-ATPase activity and decreases its activation by Cl^- at the same concentrations (10^{-7}-10^{-4} M) that via $GABA_a$ receptors activate Cl^--current in the neuronal membranes of rat hippocampus [20, 21]. The effect of this mediator on the enzyme activity was prevented by the selective blockers of $GABA_a$ receptors, picrotoxin [22] and bicuculline [23]. In addition, a cytochemical study localized the enzyme on axo- and dendrolemma, on the synaptic membranes of the inhibitory dendrodendritic $GABA_a$-synapses of fish brain [24]. Analysis of the data suggested the physiologic interaction between the Cl^--ATPase and $GABA_a$ receptors in the neuronal cell membranes. To prove the specificity of the effect on the enzyme of ligands of the receptors regulating Cl^--channels, the effects on the enzyme of ligands of different receptor proteins regulating transport of anions (Cl^-) or cations (Na^+, Ca^{2+}) should be compared. It is known that cation transport across the brain neuronal membrane of vertebrates is regulated by choline and glutamate receptors [25, 26]. Agonists of these receptors are acetylcholine and glutamate and antagonists are atropine (or benztropine mesylate) and phosphonovaleric acid, respectively [26, 27]. The Cl^--channels in neuronal membranes of animals are regulated by $GABA_a$ and glycine receptors [28]. Their agonists are GABA and glycine and antagonists are picrotoxin and strychnine, respectively. Moreover, in rat brain the $GABA_a$ receptors in contrast to glycine receptors expose binding sites for benzodiazepines (diazepam, phenazepam, etc.) and barbiturates (pentobarbital, pentobarbiton, etc.) [20, 28, 29] which are also agonists of $GABA_a$ receptors. Thus, the goal of the present study was to investigate the effects of activators and blockers of $GABA_a$/benzodiazepine, glycine, choline, and glutamate receptors on the activity of the Cl^--stimulated Mg^{2+}-ATPase of plasma membranes of bream brain.

MATERIALS AND METHODS

Bream (*Abramis brama* L.) specimens of 3-4-year age were caught in the Rybinsk reservoir and maintained in aerated aquaria under natural illumination. After decapitation, the brain was quickly removed and plasma membrane-enriched fraction was isolated as described earlier [22]. The plasma membrane fraction was stored at −30°C and subsequently used for determination of the Cl^--stimulated Mg^{2+}-ATPase activity. The ATPase activity in the plasma membrane fraction was measured by the increase in inorganic phosphorus (P_i) in 0.5 ml of an incubation medium at 30°C. The reaction was started by addition of the protein.

The enzyme activity in the presence of ligands of GABA/benzodiazepine, glycine, glutamate, and choline receptors (GABA, glycine, pentobarbital, phenazepam, acetylcholine, L-glutamic acid, benztropine mesylate, D-2-amino-5-phosphonovaleric acid, picrotoxin, strychnine) was determined as described before [22]. Membrane samples (4-10 μg) preincubated for 20 min with a ligand were added to incubation medium containing 7 mM Hepes-Tris (pH 8.4), 0.7 mM Tris-ATP, 0.7 mM $MgSO_4$, and the ligand in the same concentration as in the preincubation medium. The activity of Mg^{2+}-ATPase was calculated as the difference between

the ATPase activities in the presence and absence of $MgSO_4$. The activity of Cl^--stimulated Mg^{2+}-ATPase was determined in the presence 40 mM choline chloride, and the activation of the enzyme by Cl^- was calculated as the difference between the Mg^{2+}-ATPase activities in the presence and absence of 40 mM choline chloride in the incubation medium. To exclude the effect on the enzyme activity of ion strength of the incubation medium after addition of choline chloride, control samples contained 40 mM $NaNO_3$ instead of choline chloride [11]. Thus, the specificity of the activation of "basal" Mg^{2+}-ATPase by chloride ions was established. To exclude the possibility of hydrolysis of acetylcholine added by cholinesterases present in the membrane fraction during the preincubation, control samples instead of acetylcholine were supplemented with carbacholine (a non-hydrolyzable analog of acetylcholine) [15], which appeared to exhibit the same effect on the enzyme activity. The P_i content in samples was determined by the method of Chen [30], and total protein was determined by the Bradford method [31]. The specific activity was expressed in μmoles P_i/h per mg protein. The figures show values of the enzyme activity averaged from the results of at least three determinations. The reliability of differences between compared values was estimated by Student's *t*-test with $p < 0.05$.

GABA, Tris, and Hepes were from Bio-Rad (USA), pentobarbital from Sigma (USA), picrotoxin, benztropine mesylate, and D-2-amino-5-phosphonovaleric acid from ICN (USA), and Na_2ATP from Reanal (Hungary). Other chemicals used were domestic products.

RESULTS

The activity of the Cl^--stimulated Mg^{2+}-ATPase of plasma membranes from brain of animals consists of the "basal" Mg^{2+}-ATPase activity that can be activated by Cl^- by 40-80% [22, 32]. In the preparations studied the "basal" Mg^{2+}-ATPase activity of fish brain was 11.0 μmoles P_i/h per mg protein, and the activation of the enzyme by Cl^- was 5.4 μmoles P_i/h per mg protein (table). GABA, glycine, acetylcholine, and glutamate—mediators of $GABA_a$, glycine, acetylcholine, and glutamate receptors, respectively,—activated the "basal" Mg^{2+}-ATPase at the concentration range 10^{-7}-10^{-4} M (Fig. 1a). The maximum effect of mediators (1.3-1.8-fold increase in the enzyme activity) is observed at their concentration of 10^{-5} M, the activating effect of GABA and glycine being ~2 times higher than that of acetylcholine

Effect of mediators and blockers of receptor proteins of ion channels on the Mg^{2+}-ATPase activity in the absence and presence of 40 mM Cl^-

Ligand added	Mg^{2+}-ATPase activity, μmoles P_i/h per mg protein		
	in the absence of Cl^-	in the presence of 40 mM Cl^-	effect of Cl^-
Control	11.0 ± 0.6	16.4 ± 0.9	5.4 ± 0.6
GABA (10^{-5} M)	19.6 ± 0.7*	19.0 ± 1.2	—
GABA (10^{-5} M) + picrotoxin ($2 \cdot 10^{-5}$ M)	11.2 ± 0.5	15.7 ± 0.3	4.5 ± 0.5
Picrotoxin ($2 \cdot 10^{-5}$ M)	16.0 ± 0.8*	15.4 ± 0.9	—
GABA (10^{-5} M) + strychnine ($2 \cdot 10^{-5}$ M)	18.1 ± 1.0*	18.0 ± 0.7	—
Glycine (10^{-5} M)	17.6 ± 1.1*	16.8 ± 0.8	—
Glycine (10^{-5} M) + strychnine ($2 \cdot 10^{-5}$ M)	11.4 ± 0.9	15.5 ± 0.6	4.1 ± 0.3
Strychnine ($2 \cdot 10^{-5}$ M)	15.0 ± 1.2*	15.0 ± 0.7	—
Glycine (10^{-5} M) + picrotoxin ($2 \cdot 10^{-5}$ M)	17.5 ± 0.7*	17.3 ± 0.5	—
Acetylcholine (10^{-5} M)	14.2 ± 1.0*	19.0 ± 1.1	4.8 ± 0.5
Acetylcholine (10^{-5} M) + benztropine (10^{-5} M)	9.5 ± 0.4*	14.5 ± 0.8	5.3 ± 0.6
Benztropine mesylate (10^{-5} M)	9.0 ± 0.8*	14.0 ± 0.9	5.0 ± 0.4
Glutamate (10^{-5} M)	14.7 ± 0.9*	20.0 ± 0.6	5.3 ± 0.5
Glutamate (10^{-5} M) + D-2-amino-5-phosphonovaleric acid (10^{-5} M)	10.2 ± 0.5	15.3 ± 0.7	5.2 ± 0.7
D-2-amino-5-phosphonovaleric acid (10^{-5} M)	10.0 ± 0.8	15.0 ± 1.0	5.0 ± 0.6

Note: Values are expressed as means ± S.D. ($n = 3$).

* Significantly different from values in the medium without ligands, $p < 0.05$.

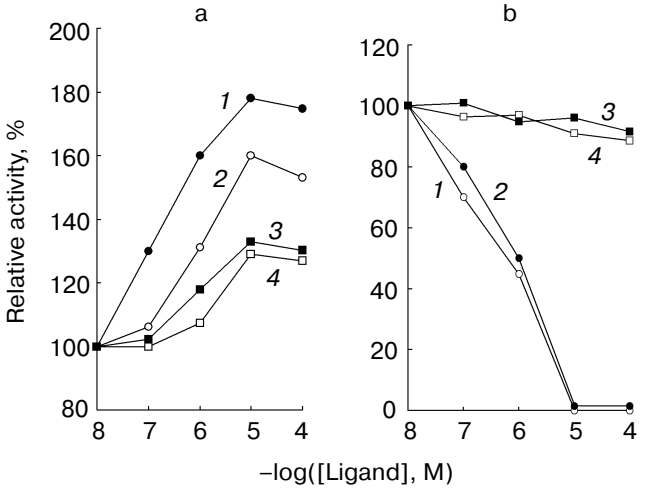

Fig. 1. Effect of GABA (*1*), glycine (*2*), glutamate (*3*), and acetylcholine (*4*) on the "basal" Mg^{2+}-ATPase activity (a) and on the activation of the Mg^{2+}-ATPase of fish brain by Cl^- (b).

and glutamate. Picrotoxin ($2\cdot10^{-5}$ M), strychnine ($2\cdot10^{-5}$ M), benztropine (10^{-5} M), and D-2-amino-5-phosphonovaleric acid (10^{-5} M) which are blockers of $GABA_a$, glycine, choline, and glutamate receptors, respectively [26-28], eliminated the activation of the "basal" Mg^{2+}-ATPase by the mediators of the corresponding receptors (table). However, in the absence of the mediators their effects on the "basal" Mg^{2+}-ATPase are different—picrotoxin and strychnine activate the enzyme activity, benztropine slightly inhibits it, and D-2-amino-5-phosphonovaleric acid does not change the enzyme activity.

The studied mediators and blockers can be separated into two groups by their effect on the activation of the "basal" Mg^{2+}-ATPase by Cl^-: ligands with (GABA, glycine, picrotoxin, strychnine) and without effect on the activation (acetylcholine, glutamate, benztropine, D-2-amino-5-phosphonovaleric acid). Thus GABA and glycine suppress the activation of the enzyme by Cl^- in the concentration-dependent manner and at concentrations 10^{-5}-10^{-4} M completely eliminate it (table, Fig. 1b). The inhibiting effect of these mediators is prevented by the blockers picrotoxin and strychnine, respectively (table). In the absence of the mediators, these blockers eliminate the activation of the enzyme by Cl^- (table). Acetylcholine and glutamate have no effect on the activation of the "basal" Mg^{2+}-ATPase by Cl^- (table, Fig. 1b). The blockers benztropine and D-2-amino-5-phosphonovaleric acid both in the presence and absence of the corresponding mediators also have no effect on the activation of the enzyme by Cl^- (table).

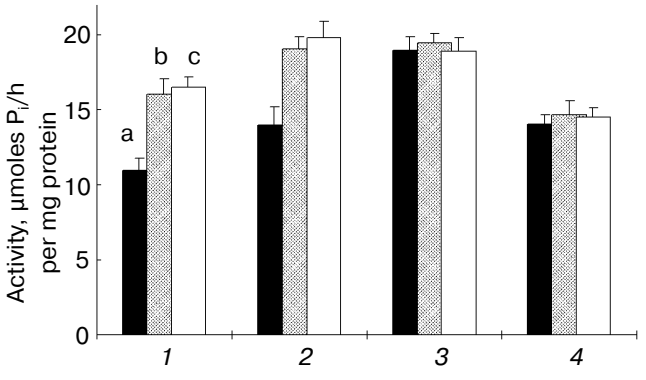

Fig. 2. Effect of $5\cdot10^{-7}$ M phenazepam (b) and 10^{-5} M pentobarbital (c) on the "basal" Mg^{2+}-ATPase activity (a) of fish brain in the control (*1*) and in the presence of 10^{-7} M GABA (*2*), 10^{-4} M GABA (*3*), or 10^{-6} M glycine (*4*).

Previously it was shown that picrotoxin (10^{-6} M) inhibits both GABA- and glycine-induced Cl^--current, whereas strychnine (10^{-6} M) inhibits only the glycine-induced ion current across rat brain receptors expressed in the oocytes of *Xenopus laevis* [28]. Therefore we studied the specificity of the effect of these blockers on the stimulation of the enzyme activity by GABA and glycine. For this purpose, GABA was introduced into the preincubation medium simultaneously with the non-corresponding blocker strychnine, and glycine simultaneously with picrotoxin. It was found that strychnine ($2\cdot10^{-5}$ M) has no effect on the activation of the "basal" Mg^{2+}-ATPase by GABA (10^{-5} M) as well as picrotoxin ($2\cdot10^{-5}$ M) has no effect on its activation by glycine (10^{-5} M) (table). Moreover, in such combinations with mediators the blockers do not "cancel" their inhibiting effect on the enzyme activation by Cl^- (table).

It was shown with rat hippocampus neuronal culture that pentobarbital (10^{-5}-10^{-4} M) [20] and diazepam (10^{-6}-10^{-5} M) [33] increase the GABA (10^{-6} M)-induced Cl^--current. Therefore, we studied the effects of phenazepam ($5\cdot10^{-7}$ M) and pentobarbital (10^{-5} M) on the GABA- or glycine-activated "basal" Mg^{2+}-ATPase activity. The effects of these ligands on the enzyme activity significantly vary depending on the type of mediator used and its concentration. Thus, phenazepam and pentobarbital stimulate the activation of the Mg^{2+}-ATPase by low concentrations of GABA (10^{-7} M) and have no effect on the enzyme activity in the presence of high GABA concentration (10^{-4} M). In addition, the ligands used do not affect the activation of the "basal" Mg^{2+}-ATPase activity by glycine (10^{-6} M) (Fig. 2).

It was shown by electrophysiological studies that barbiturates in the absence of mediators activate the Cl^--current across $GABA_a$/benzodiazepine receptors of rat hippocampal neurons [20, 33]. In our studies in the absence of mediators, phenazepam in the concentration range 10^{-8}-10^{-4} M and pentobarbital in the concentration range 10^{-6}-10^{-3} M increase the activity of the "basal" Mg^{2+}-ATPase (Fig. 3). The dependence of the enzyme

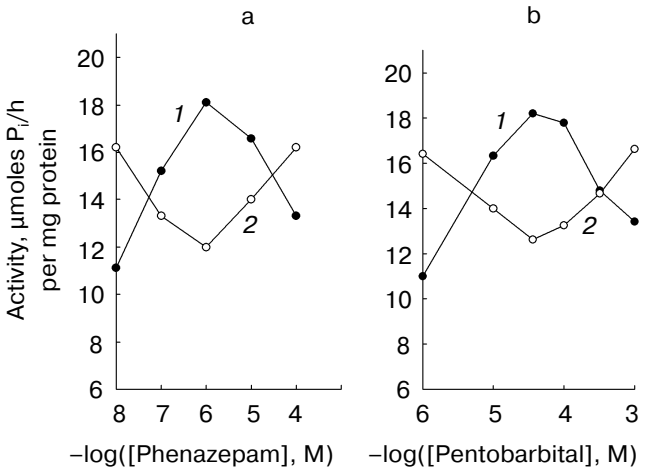

Fig. 3. Effect of phenazepam (a) and pentobarbital (b) on the Mg^{2+}-ATPase activity of fish brain in the absence (*1*) and presence (*2*) of 40 mM Cl^-.

activity on ligand concentration is bell-shaped. The maximum effect (1.7-fold increase of the enzyme activity) is observed at 10^{-6} M phenazepam and $5 \cdot 10^{-5}$ M pentobarbital. In the presence of Cl^- the dependence of the enzyme activity on concentration of these ligands also is bell-shaped, but in this case the ligands inhibit the Cl^--stimulated Mg^{2+}-ATPase activity (Fig. 3). The maximum inhibition of the enzyme (1.3-fold decrease of the enzyme activity) is observed at 10^{-6} M phenazepam and $5 \cdot 10^{-5}$ M pentobarbital. Thus both phenazepam and pentobarbital, similar to GABA and glycine, activate the "basal" and decrease the Cl^--stimulated Mg^{2+}-ATPase activities.

DISCUSSION

On comparison of the effects of mediators studied on the activity of the "basal" Mg^{2+}-ATPase of the plasma membranes of fish brain, a similar pattern was revealed, i.e., all mediators activate the enzyme with maximum effect at 10^{-5} M concentration, and activation is removed by blockers of the corresponding receptors. The concentrations of mediators which activate the "basal" Mg^{2+}-ATPase are efficient also for the corresponding ligand-regulated receptor proteins. Thus, it was shown using electrophysiological methods that GABA (from $2 \cdot 10^{-5}$ to $3 \cdot 10^{-4}$ M), glycine (from 10^{-4} to $2 \cdot 10^{-3}$ M), and glutamate (from 10^{-5} to 10^{-3} M), when acting on the corresponding receptors of rat brain expressed in *Xenopus laevis* oocytes, increase the ion conductivity of the membrane [28], and acetylcholine (from 10^{-5} to 10^{-3} M) via choline receptors enhances the membrane current in the abdominal neuron of guinea pig [25].

In the absence of the mediators the effects of the blockers on the "basal" Mg^{2+}-ATPase differ, i.e., benztropine and D-2-amino-5-phosphonovaleric acid have practically no effect on the "basal" Mg^{2+}-ATPase activity whereas picrotoxin and strychnine activate it. Previously in the study of the effect on the activity of the "basal" Mg^{2+}-ATPase of another selective blocker of the $GABA_a$ receptors, bicuculline [28], it was shown that low (from 10^{-6} to $2 \cdot 10^{-5}$ M) and high concentrations of the blocker (from $2 \cdot 10^{-5}$ to 10^{-4} M) activate and inhibit the enzyme, respectively [23]. This property of ligands to appear as agonists or antagonists depending on conditions of incubation and their concentration is observed in studies of not only the kinetics of allosteric enzymatic reactions [34], but also of ligand-regulated ion channels. Thus, it was shown in electrophysiological studies on recombinant $GABA_a$/benzodiazepine receptors of rat brain expressed in fibroblast culture that bicuculline can behave as an invert agonist and cause the activation of Cl^--current across cell membranes instead of inhibition at the concentration of 10^{-3} M [35].

Significant differences between ligands regulating cation or anion transport were revealed on study of their effects on the activation of the "basal" Mg^{2+}-ATPase by Cl^-. Thus, the mediators of choline and glutamate receptors and their antagonists the benztropine and D-2-amino-5-phosphonovaleric acid do not affect this enzyme activity [26, 27], while the mediators of GABA and glycine receptors suppress the activation of the "basal" Mg^{2+}-ATPase by Cl^-. The antagonists of these receptors, picrotoxin and strychnine [28], also suppress the activation of the "basal" Mg^{2+}-ATPase by Cl^- and, moreover, "cancel" its inhibition by GABA and glycine. The results obtained support our earlier suggestion that in the membranes of fish brain the Cl^--stimulated Mg^{2+}-ATPase activity interacts with $GABA_a$ and glycine receptors and does not interact with choline and glutamate receptors [36].

Comparing the data shown in the table and Fig. 1 (a and b), we can see that in the presence of GABA- and glycine-ergic compounds the increase in the "basal" Mg^{2+}-ATPase activity is accompanied by a decrease of its activation by Cl^-. Previously on study of properties of the Cl^--stimulated Mg^{2+}-ATPase we found such conditions (high concentration of the protein and Mg^{2+}-ATP) which prevent the enzyme stimulation by Cl^- [37]. Under these conditions GABA and glycine and also picrotoxin and strychnine, in contrast, inhibit the "basal" Mg^{2+}-ATPase activity and do not mask its activation by Cl^-. The revealed coupling of the "basal" Mg^{2+}-ATPase activity and its activation by Cl^- when affected by these ligands is rather due to the fact that both enzyme activities are manifested by the same enzyme, i.e., the Cl^--stimulated Mg^{2+}-ATPase. Therefore, if in the presence of activator or blocker the activity of the "basal" Mg^{2+}-ATPase achieves high levels when the molecular turnover is maximal [38], then an additional activation of the enzyme by Cl^- cannot take place. The same phenomenon seems to

be observed on addition of pentobarbital and phenazepam to the "basal" Mg^{2+}-ATPase activated by high concentration of GABA (10^{-4} M)—additional activation of the enzyme by the ligands is not possible in the presence of high concentrations of the mediator providing the maximum value of the enzyme activity. While phenazepam and pentobarbital stimulate the "basal" Mg^{2+}-ATPase activated by low concentrations of GABA (10^{-7} M), i.e., these ligands show synergism. Previously it was shown that pentobarbital (from 10^{-4} to 10^{-3} M) enhances the GABA (10^{-5} M)-induced uptake of $^{36}Cl^-$ by synaptoneurosomes of rat brain [39], and diazepam ($5\cdot10^{-6}$ M) increases the GABA ($2\cdot10^{-6}$ M)-induced Cl^--current in neuronal cells of rat hippocampus [33]. Moreover, in the presence of $5\cdot10^{-5}$ M pentobarbital or chlorazepat (a derivative of benzodiazepine), the GABA (10^{-5} M)-induced ionic conductivity of membranes of *Xenopus laevis* oocytes increased approximately 2-fold, whereas the glycine-induced ionic conductivity did not change [28]. In our study, pentobarbital and phenazepam also have no effect on the activation of the "basal" Mg^{2+}-ATPase by glycine. These data indicate the similarity of the ligand effects on the Mg^{2+}-ATPase activity of plasma membranes from fish brain and on the $GABA_a$/benzodiazepine and glycine receptor proteins, the concentrations of the agonists and antagonists used in our study being close to the concentrations effective toward the given receptors.

In the present work the activation of "basal" Mg^{2+}-ATPase by phenazepam and pentobarbital is described by a bell-shaped curve that suggests a complex influence of these ligands on the enzyme [34]. The effect of pentobarbital on the enzyme activity is similar to its effect on the Cl^--current in the frog sensory neurons [40] and also on the $^{36}Cl^-$ uptake by brain synaptoneurosomes of rat [39] and fish [41]. Thus, in the absence of GABA, pentobarbital (from 10^{-5} to 10^{-3} M) acts as an allosteric agonist showing the two-phase effect on the uptake of $^{36}Cl^-$ by fish brain synaptoneurosomes—it increases the $^{36}Cl^-$ uptake by synaptoneurosomes at low concentrations (from 10^{-5} to $5\cdot10^{-4}$ M) with the maximum effect at $3\cdot10^{-4}$ M and decreases it to initial value [41] at high concentrations (from 10^{-3} to $5\cdot10^{-3}$ M). It was established that high concentrations of pentobarbital via an allosteric interaction with a $GABA_a$ receptor cause its desensitization, thus influencing the Cl^- transport [39]. In our study the absence of the activating effect of high concentrations of pentobarbital or phenazepam on the "basal" Mg^{2+}-ATPase activity is possibly related also with conformational changes of the receptor. The higher sensitive of the enzyme to pentobarbital observed in our experiments is most probably due to the preincubation of the plasma membranes with the ligand (the authors of the cited above work did not use this approach). This suggestion is supported by the data on the enhanced effect of pentobarbital on the $^{36}Cl^-$ uptake by synaptoneurosomes of rat brain after their preincubation with this ligand [39]. The inhibition of the Cl^--stimulated Mg^{2+}-ATPase by phenazepam and pentobarbital can be influenced by the ionic strength of the incubation medium containing 40 mM choline chloride. Thus, it was shown that addition of 50 mM NaCl to the physiological medium increases by 50% the affinity of ^3H-labeled diazepam binding to mice brain membranes [42]. The decrease in the Cl^--stimulated Mg^{2+}-ATPase activity coupled with the increase in the "basal" Mg^{2+}-ATPase activity can be partly due to the approaching the limiting value of the enzyme activity when the enzyme molecular turnover is maximal.

Thus, the data obtained show that the ligands of receptor proteins regulating both cation and anion channels affect the activity of the "basal" Mg^{2+}-ATPase of the plasma membranes of fish brain. However only the activators and blockers of the ligand-regulated Cl^--channels change the activity of the Cl^--stimulated Mg^{2+}-ATPase that is, possibly, related with its Cl^--transporting function. It is known that GABA and glycine regulate Cl^- transport via $GABA_a$ and glycine receptors [28] whereas glutamate and acetylcholine—mediators, respectively, of glutamate and choline receptors—regulate to a greater extent the transport of Na^+ or Ca^{2+} [25, 26]. This is possibly the reason why they act only on the "basal" Mg^{2+}-ATPase and do not affect its activation by Cl^-. The functional significance of changes of the Cl^--stimulated Mg^{2+}-ATPase activity in response to the ligands of the inhibitory receptors is not yet clear. However, if the Cl^--stimulated Mg^{2+}-ATPase provides the active transport of chloride ions [11], one can suggest that the ligands of $GABA_a$/benzodiazepine and glycine receptors via the receptor-dependent pathway participate in the specific regulation of the ATP-dependent Cl^- transport across the neuronal cell membranes.

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