Sensitivity of the Brain Synaptosomal Membrane Mg^{2+}-ATPase Activity to Arachidonic Acid is under Control of the Na^+,K^+-ATPase State

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Abstract. Evidence is presented for the sensitivity of the synaptosomal plasma membrane Mg^{2+}-ATPase activity to arachidonic acid being dependent on the functional state of Na^+,K^+-ATPase. An "Inversion effect" was observed at arachidonic acid concentrations exceeding 80 μmol/l when the Mg^{2+}-ATPase activity (after ouabain addition) is higher than the total ATPase activity (without ouabain). The "Inversion effect" is reduced by cyclooxygenase inhibitor indomethacin or acetylsalicylic acid and restored by prostaglandin PGA_2 or PGD_2.

Key words: Mg^{2+}-ATPase — Na^+,K^+-ATPase — Arachidonic acid — Synaptosomal membranes

Introduction

It has been recently recognized that arachidonic acid metabolites can play a role of a second messengers similarly as do cyclic nucleotides, calcium, diacylglycerol and inositolpolyphosphates (Shimizu et al. 1979; Bevan and Wood 1987; Piomelli et al. 1987). The intramembrane free arachidonic acid levels are under control of phospholipases A_2 and C and diacylglycerolipase activities. The content of free arachidonic acid in biological membranes was shown to increase upon receptor-mediated stimulation of these enzymes (Axelrod et al. 1988; Dumnis et al. 1990) and under several pathologies such as ischemia (Katz et al. 1976) and hypoxia (Tada et al. 1978).

Free unsaturated fatty acids, including arachidonic acid, are responsible for multiple functional disorders in cells of the nervous tissue such as inactivation of the muscarinic cholinergic receptors (Aronstam et al. 1977; Okun et.al. 1986*a*), blockade of sodium-dependent transport systems for aminoacids and neuromediators (Chan et al. 1983; Rhoads et al. 1983) and of Na^+,K^+-ATPase (Ahmed and Thomas 1971; Swann 1984).

Na^+,K^+-ATPase and Mg^{2+}-ATPase have major share on ATP-hydrolysing activities of synaptosomal membranes in calcium-free medium, the ATP binding site of the former being localised on the inner membrane surface, and that of the latter on the outer surface (Sorensen and Mahler 1982; Nagy et al. 1986). There is experimental evidence for Na^+,K^+-ATPase and Mg^{2+}-ATPase of the neuronal plasma membrane being different enzymes (Tanaka 1974) rather than different catalytic states of the same molecule.

The present work deals with the investigation of the influence of arachidonic acid on ATPase activities of rat brain synaptosomal membranes. Na^+ K^+-ATPase "switching off" by ouabain or by omitting of sodium or potassium ions from bathing medium is shown to change the sensitivity of the Mg^{2+}-ATPase activity to arachidonic acid, the effect being controlled by prostaglandins.

Materials and Methods

Synaptosomal membranes were isolated from the hemispheres of albino rat brains in accordance with Jones and Matus (1974). Membrane pellets were resuspended in bidistilled water at a protein concentration of about 8–10 mg/ml and stored at −18 °C up to 6 days. The total ATPase activity (in the absence of ouabain) and the Mg^{2+}-ATPase activity (in the presence of 0.5 mmol/l ouabain) was measured as P_i release rate (Fiske and Subbarow 1925) in incubation medium *A*, containing (in mmol/l): NaCl, 100; KCl, 20; $MgCl_2$, 3; Tris-HCl, 30 (ph 7.5). In some experiments the Mg^{2+}-ATPase activity was measured in a medium devoid of ouabain with choline ions equimolarly substituted for potassium (medium *B*) or sodium (medium *C*) ions. It should be noted that the levels of Mg^{2+}-ATPase activity in control samples treated with ouabain did not differ significantly from those measured in the absence of Na^+, K^+ or both of them. The reaction was started with addition of 0.05 ml ATP-Na_2 (60 mmol/l) to 0.95 ml of the membrane suspension (protein concentration 50 μg/ml) preincubated for 40 min with different additives at 37 °C, and incubation was stopped after 20 minutes by mixing with equal volume of 20% trichloroacetic acid. With medium *C*, Tris-ATP was used. The Na^+,K^+-ATPase activity was not affected by preincubation times between 10–60 minutes at 37 °C. In all experiments described P_i release was linear over time of incubation with ATP. Free fatty acids in ethanol solutions were injected into membrane suspension at the 20th minute of preincubation. The final concentration of ethanol was 1% in all samples including those without arachidonic acid; at this concentration ethanol does not influence the ATPase activity (see also Nhamburo et al. 1987). Other additions were made at the 5th minute of preincubation.

Results

Characteristics of Mg^{2+}-ATPase activity measured in our experiments were compared with that of Mg^{2+}-dependent ecto-ATPase reported by Nagy et al. (1986). The substrate specificity of Mg^{2+}-ATPase activity was determined in the same medium as that used for ATP as substrate, by substituting various nucleotides

for ATP. The results are presented in Table 1. At the nucleotide concentration tested (3 mmol/l) and at nucleotide-to-magnesium ratio of 1 : 1, the enzyme

Table 1. Enzymatic activities of synaptosomal membrane Mg^{2+}-ATPase with various substrates (μmol P_i/mg h)

Substrate	Activity
ATP	13.7 ± 1.2
GTP	15.5 ± 1.6
UTP	10.3 ± 1.4
CTP	9.7 ± 1.3
ADP	1.3 ± 0.2
AMP	0.1 ± 0.2
p-Nitrophenylphosphate	0.1 ± 0.1

Each value is the mean of three determinations ± S.E.M.

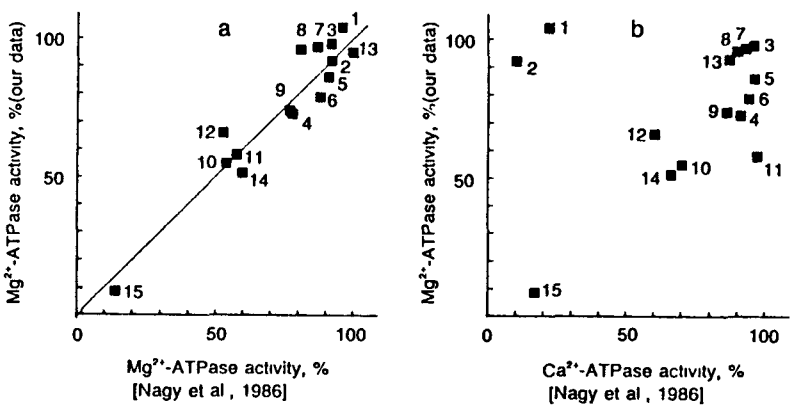

Figure 1. The synaptosomal membrane Mg^{2+}-ATPase activity of the present work vs. the synaptosomal Mg^{2+}-ATPase (Fig. 1*a*) or Ca^{2+}-ATPase (Fig. 1*b*) activity measured by Nagy et al. (1986, Table 6) in the presence of various inhibitors: 2,4-dinitrophenol, 1 – 0.01 mmol/l, 2 – 0.1 mmol/l; oligomycin, 3 – 0.2 μg/ml, 4 – 2.0 μg/ml; N-ethylmaleimide, 5 – 0.01 mmol/l; 6 – 0.1 mmol/l; sodium azide, 7 – 0.01 mmol/l, 8 – 0.1 mmol/l; dicyclohexylcarbodiimide, 9 – 0.01 mmol/l, 10 – 0.1 mmol/l; *p*-chloromercurybenzoate, 11 – 0.01 mmol/l, 12 – 0.1 mmol/l; trifluoroperazine, 13 – 0.01 mmol/l, 14 – 0.1 mmol/l, 15 – 1.0 mmol/l. The activity is expressed as percentage of the control enzymatic activity measured in the absence of the inhibitors. Control Mg^{2+}-ATPase activity was about 14 μmol P_i/mg h. The values shown are means of triplicate determinations. The correlation line was drawn by the least square fitting.

activities increased in the order ATP = GTP > UTP = CTP > ADP > AMP = *p*-nitrophenylphosphate. This is in good agreement with the data of Nagy et al. (1986).

Moreover, the sensitivities of Mg^{2+}-ATPase activity and that of the synaptosomal Mg^{2+}-dependent ecto-ATPase (data from Table 6 of Nagy et al. (1986)) to various inhibitors were compared. A good correlation ($r = 0.95$) was shown between our results and those of Nagy and coworkers, the regression line having a slope of 1.02 ± 0.09 as calculated by least square fitting (Fig. 1*a*). In contrast, there was no correlation between the sensitivity to inhibitors of Mg^{2+}-ATPase activity in the present work and that of another ecto-ATPase, Ca^{2+}-ATPase, measured by Nagy and coworkers (1986) (Fig. 1*b*; correlation coefficient is as small as 0.28).

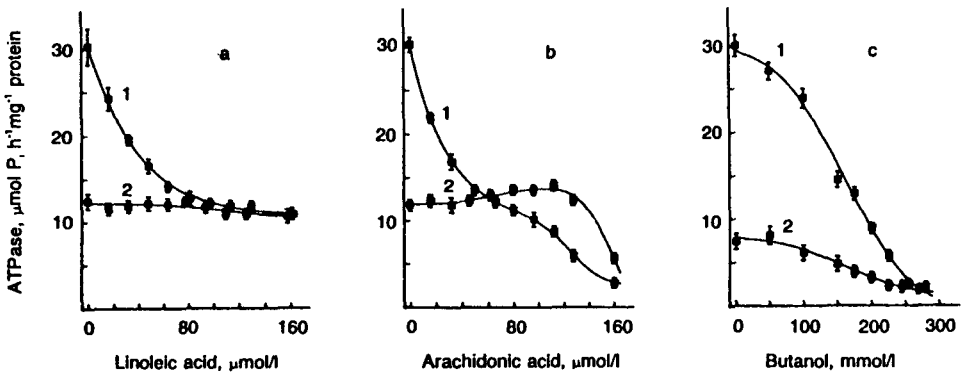

Figure 2. Influence of polyunsaturated free fatty acids (*a* – linolenic, *b* – arachidonic) and butanol (*c*) on total ATPase (1) and Mg^{2+}-ATPase (2) activities in rat brain synaptosomal membranes. Means ± S.E.M. are presented of one typical experiment measured in triplicate.

Fig. 2 shows inhibition of total ATPase and Mg^{2+}-ATPase activities of synaptosomal membranes with linolenic acid (*a*), arachidonic acid (*b*) and butanol (*c*). Obviously, linolenic acid has no effect on the Mg^{2+}-ATPase activity and inhibits the Na^+,K^+-ATPase so that the total activity drops to the Mg^{2+}-ATPase activity level. With arachidonic acid as a perturber, the picture is more complicated. At arachidonic acid concentrations exceeding 60 μmol/l, there is a minor stimulation of the Mg^{2+}-ATPase activity with a subsequent inhibition phase, the extent of the stimulation varying from sample to sample. The total ATPase activity is inhibited over the entire range of arachidonic acid concentrations tested, and above 80 μmol/l the activity becomes oven lower than that of Mg^{2+}-ATPase i.e. the "Inversion effect" takes place. The pattern of the inhibition of ATPases with butanol

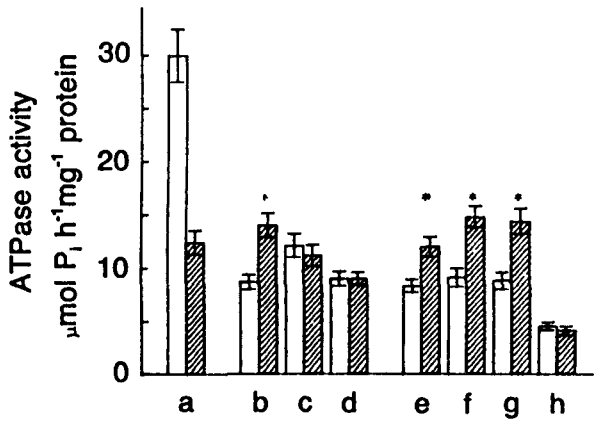

Figure 3. The total ATPase (without ouabain, open bars) and Mg^{2+}-ATPase (with 0.5 mmol/l ouabain, closed bars) activities as a function of the rat brain synaptosomal membrane modification with different additives: *a* – in the absence of any additive; *b* – arachidonic acid (110 μmol/l); *c* – indomethacin (0.1 mmol/l) + arachidonic acid; *d* – acetylsalicylic acid (0.1 mmol/l) + arachidonic acid; *e* – acetylsalicylic acid + arachidonic acid + PGD_2(10 nmol/l); *f* – acetylsalicylic acid + arachidonic acid + PGA_2(10 nmol/l); *g* – acetylsalicylic acid + linoleic acid (110 μmol/l) + PGA_2; *h* – butanol (250 mmol/l) + PGA_2. Increments of Mg^{2+}-ATPase activity over that of total ATPase activity significant at $p < 0.05$ are marked with asterisks. Mean values ± S.E.M. are presented for three experiments, three determinations each.

is similar to that observed for linolenic acid but with somewhat more pronounced suppression of the Mg^{2+}-ATPase activity.

Fig. 3 summarizes data concerning the influence of inhibitors and metabolites of the cyclooxygenase metabolic pathways of polyunsaturated fatty acids on the "Inversion effect". Column (*a*) represents the total (control) ATPase and ouabain-insensitive, Mg^{2+}-ATPase, activities, the latter being about 3 times lower than the total activity. Arachidonic acid (110 μmol/l) inhibits total activity but tends to stimulate the Mg^{2+}-ATPase so that the "Inversion effect" may be seen (column *b*). The cyclooxygenase inhibitor indomethacin (column *c*) or acetylsalicylic acid (column *d*) at 0.1 mmol/l abolish the "Inversion effect". Added together with acetylsalicylic acid, PGD_2 (10 nmol/l, column *e*) or PGA_2 (10 nmol/l, column *f*) recovers the arachidonic acid-induced inversion of the ATPase activities. In the presence of PGA_2, linolenic acid (110 μmol/l) also exhibits the "Inversion effect" (column *g*). No "Inversion effect" was observed with butanol alone (Fig. 2*c*) or in combination with PGA_2 (Fig. 3, column *h*).

Table 2 shows data suggesting an influence of arachidonic acid on the Mg^{2+}-ATPase activity, upon "switching off" the Na^+,K^+-ATPase with ouabain or by

Table 2. Relative ATPase activities of rat brain synaptosomal membranes in the presence of arachidonic aid (110 μmol/l) in different media.

Conditions	Activity (%)
Total ATPase	
Medium A	100 ± 7 ($n = 6$)
Mg^{2+}-ATPase	
Medium *A* + ouabain	162 ± 13 ($n = 2$)
Medium *B* (potassium-freee)	142 ± 12 ($n = 2$)
Medium *C* (sodium-free)	140 ± 12 ($n = 2$)

For the composition of the media, see "Materials and Methods". Activity in medium *A* in the presence of arachidonic acid is taken for 100% ($8.7 \pm 0.6\mu$mol P_i/mg h). Mean values $\pm$ S.E.M. are given; figures in the parentheses are numbers of experiments, each measured in triplicate.

omission of sodium or potassium ions from the incubation medium, as compared with the total ATPase activity measured in medium *A* with arachidonic acid as the only addition. There is statistically significant prevalence of variants with the Na^+,K^+-ATPase in "switched off" state. The prevalence does not practically depend on how Na^+,K^+-ATPase has been blocked.

Discussion

In synaptosomal membranes, several ATPases were identified including transport ($Na^+ + K^+$)-ATPase and ($Ca^{2+} + Mg^{2+}$)-ATPase and nontransport Mg^{2+}-ATPase and Ca^{2+}-ATPase (Sorensen and Mahler 1982), the ATP hydrolysis sites of transport ATPases facing the synaptoplasma and those of nontransport ones being oriented at the junctional side of the presynaptic membrane (ecto-ATPases) (Sorensen and Mahler 1982; Nagy et al. 1986). To identify the Mg^{2+}-ATPase activity under study, its substrate specificity (Table 1) and its sensitivity to different inhibitors (Fig. 1*a*, *b*) were compared with those of Mg^{2+}-ATPase and Ca^{2+}-ATPase well documented by Nagy et al. (1986). In a good accordance with the data of these authors, the substrates most susceptive to Mg^{2+}-ATPase activity were nucleotide triphosphates while AMP and *p*-nitrophenylphosphate were not metabolized in our assay system. It can be inferred that the Mg^{2+}-ATPase activity belongs to ATP hydrolysing enzyme rather than to a phosphatase. On the other hand, the lack of marked differences in the rates of hydrolysis of various nucleotide triphosphates is a strong point against the Mg^{2+}-ATPase activity measured reflecting some modified form of Na^+,K^+-ATPase. Indeed, the latter shows a strong specificity towards

ATP, other nucleotides being hydrolyzed at much lower rates (from 15% to 0.5% of the activity with ATP) (Stekhoven and Bonting 1981). Another argument is the absence of any differences in values of the Mg^{2+}-ATPase activity measured with ouabain or after omission of K^+, Na^+ or of both of them. However, our data do not allow to distinguish between Mg^{2+}-ATPase and Ca^{2+}-ATPase because of their nearly identical substrate specificity (Nagy et al. 1986). More unequivocal results in this respect were obtained from a comparison of the ATPase susceptibility to inhibitors (Fig. 1). A close correlation with a regression coefficient close to 1 is evident between magnitudes of inhibition of Mg^{2+}-ATPase activity measured by us and by Nagy et al. (1986). No such correlation exists between Mg^{2+}-ATPase inhibition in the present work and data on Ca^{2+}-ATPase inhibition measured by Nagy et al. (1986) (Fig. 1*b*). The results support the conclusion that the Mg^{2+}-ATPase activity measured in our conditions reflects Mg^{2+}-ATPase as a separate enzyme.

It follows from the present results that the Na^+,K^+-ATPase of synaptosomal membranes is strongly inhibited by arachidonic acid, in full accordance with the data of Vyskočil et al. (1987). However, unlike these authors we failed to notice any activation of Na^+,K^+-ATPase within the arachidonic concentration range tested. This discrepancy is possibly due to the fact that activation is observed only at suboptimal ATP or potassium concentration which is not our case.

In this paper, evidence is presented for an existence of an interaction between Na^+,K^+-ATPase and Mg^{2+}-ATPase in rat brain synaptosomal membranes. The interaction is visualized through modification of the Mg^{2+}-ATPase sensitivity to arachidonic acid being dependent upon the functional state (active/nonactive) of Na^+,K^+-ATPase. Upon inactivation of Na^+,K^+-ATPase with ouabain, arachidonic acid induces minor stimulation of Mg^{2+}-ATPase at lower concentrations with subsequent inhibition of it at higher concentrations (Fig. 2*b*). A similar influence of unsaturated fatty acid was shown for the Mg^{2+}-ATPase of human erythrocyte membranes (Schmalzing and Kutschera 1982). Figure 2*a* shows Na^+,K^+-ATPase to be totally inhibited at polyunsaturated fatty acid levels higher than 80μmol/l, and events at higher concentrations are mainly related to the Mg^{2+}-ATPase activity. Hence the "Inversion effect" (i.e. a prevalence of Mg^{2+}-ATPase compared to total ATPase activity) may reflect a modification of the Mg^{2+}-ATPase sensitivity to arachidonic acid as a result of a change in the Na^+,K^+-ATPase functional state: upon addition of arachidonic acid to the membranes with the Na^+,K^+-ATPase in the active state (without ouabain), Mg^{2+}-ATPase activity becomes lower than with the Na^+,K^+-ATPase being inactivated with ouabain. Nevertheless, it may be suggested that the effect observed is caused by a direct action of ouabain controlling the Mg^{2+}-ATPase sensitivity to arachidonic acid through an unknown mechanism. The data in Table 2, however, exclude such a possibility because nearly the same "Inversion effect", i.e. an increment of Mg^{2+}-ATPase over total ATPase, is ob-

served when omission of sodium or potassium ions instead of ouabain is used to block Na^+,K^+-ATPase activity. It is known (Skou 1990) that the presence of either sodium or potassium ions shifts Na^+,K^+-ATPase to E_1 or E_2 conformation, respectively. Thus, a shift in $E_1 \rightleftharpoons E_2$ equilibrium may be responsible for the modulation of the Mg^{2+}-ATPase sensitivity to arachidonic acid.

As for the mechanisms of the relationship between Na^+,K^+-ATPase and Mg^{2+}-ATPase, it should be noted that neither linolenic acid nor butanol exhibit the "Inversion effect" (Fig. 2). Thus, it can be supposed that specific metabolites of arachidonic acid are involved. Indeed, Figure 3 shows that the prostaglandin synthesis inhibitors, indomethacin or acetylsalicylic acid, abolish the "Inversion effect", but PGA_2 or PGD_2 restore it. It should be noted that the restoration of the interenzyme "relationship channel" occurs at a physiologically low concentration of the prostaglandins (10 nmol/l), at which neither Na^+,K^+-ATPase nor Mg^{2+}-ATPase are affected. The influence of PGA_2 and PGD_2 on these enzymes was only observed in the micromolar concentration range (Gilbert and Wyllie 1975; Shibata et al. 1982; Deliconstantinos 1986). We conclude that arachidonic acid acts as a modulator of Na^+,K^+-ATPase and Mg^{2+}-ATPase on the one hand, and as a prostaglandin precursor controlling the interenzyme relationship on the other hand.

The modulation of the enzymatic activities with arachidonic acid can be linked to changes either in the physical state of the lipid microenvironment (Okun et al. 1986*a*), or in density of electric charges near the enzyme molecules upon segregation of the negatively charged lipid molecules (Brotherus et al. 1980). The evidence in favour of the latter mechanism is that the "Inversion effect" is also observed with linolenic acid in the presence of the prostaglandins (Fig. 3*g*) but not with neutral butanol (Fig. 3*h*), the latter having the same effect on the annular and bilayer lipid microviscosity measured with pyrene (Okun et al. 1986*b*) as does arachidonic acid (Okun et al. 1986*b*) as does arachidonic acid (Okun et al. 1986*a*) (data not shown).

Our experimental data seem to be the first evidence for the existence of a relationship between the Na^+,K^+-ATPase and the Mg^{2+}-ATPase in brain synaptosomal membranes. The link between the Na^+,K^+-ATPase functional state, modulated with ouabain, and chemosensitivity of the acetylcholine receptors was shown earlier in molluscan neurons (Ayrapetyan and Arvanov 1979) Unfortunately, the Mg^{2+}-ATPase function in synaptosomal membranes is unknown to date. However, such a type of cell membrane systems regulation may be of a more general character, and molecular events which lead to the coupling of Na^+,K^+-ATPase with other functional protein molecules are still to be studied.

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