Biochimica et Biophysica Acta, 1069 (1991) 127–130

ADONIS 000527369100373P

BBAMEM 70692

Rapid Report

A second messenger role for monoacylglycerols is suggested by their activating effects on the sodium pump

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(Received 2 August 1991)

Key words: Sodium pump; ATPase, Na^+/K^+-; Diacylglycerol; Monoacylglycerol

Receptor-mediated activation of the sodium pump has been noted in several intact tissues. To test the hypothesis that this may be due to the direct effects of the second messenger diacylglycerols on the pump, we studied the effects of various long-chain acylglycerols on the purified Na^+/K^+-ATPase. With optimal ATP, acylglycerols had no effect on enzyme activity. When ATP was suboptimal, tri- and diacylglycerols had no effects, but monoacylglycerols caused up to 3-fold increase in ATPase activity. Using sealed vesicles of red cell membranes and cardiac sarcolemma, stimulation of the ion transport function of the enzyme by monoacylglycerols in the presence of suboptimal ATP was also shown. Since the sodium pump may not be saturated with ATP in the intact cell, the possibility arises that monoacylglycerols are the second messengers for the receptor-mediated regulation of the pump.

Na^+/K^+-ATPase (i.e., the sodium pump) is the intrinsic enzyme of the plasma membrane that carries out the coupled active transport of Na^+ and K^+ in most eucaryotic cells. In several intact tissues and cells, various hormones and neurotransmitters have been shown to cause receptor-mediated stimulation of the sodium pump activity [1–7]. Because in many such situations the same receptor activation that stimulates the pump is also known to increase the rate of hydrolysis of phosphatidylinositol to inositol 1,4,5-trisphosphate and diacylglycerol, the possibility has been considered that pump stimulation may be caused through the activation of protein kinase C by diacylglycerol; followed either by a kinase-induced rise in intracellular Na^+ leading to increased pump rate, or by a more direct regulatory effect of protein kinase C on Na^+/K^+-ATPase [2–7]. Receptor-mediated pump stimulation secondary to increased intracellular Na^+ concentration has been ruled out in several studies [4,6]. Also, while the phosphorylation of the α-subunit of Na^+/K^+-ATPase by protein kinase C has been demonstrated [8], to date no evidence for a functional significance of this reaction has been reported. Recent studies of our laboratory on the reaction mechanism of Na^+/K^+-ATPase have indicated the existence of a hydrophobic regulatory site of the enzyme, the occupation of which by some hydrophobic ligands increases the enzyme activity when ATP is suboptimal [9–11]. In view of this, it occurred to us that receptor-linked activation of the sodium pump may be due to the direct interactions of diacylglycerols with Na^+/K^+-ATPase rather than through protein kinase C. To test this possibility, we examined the effects of several long-chain acylglycerols on the hydrolytic and the transport functions of Na^+/K^+-ATPase.

The purified membrane-bound enzyme with the specific activity of 1000–1500 μmol of ATP hydrolyzed/mg per h, was prepared from canine kidney medulla [12]. Assay of ATPase activity was done at 37 °C through the determination of the initial rate of release of [$^{32}P_i$] from [γ-^{32}P]ATP [12]. Reaction mixtures for this assay contained 100 mM NaCl, 25 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, the indicated concentrations of ATP (added with an equal concentration of Mg^{2+}), the indicated concentrations of fatty acid derivatives added as solutions or sonicated suspensions, 0.2–0.5 μg of enzyme protein/ml, 25 mM Tris, and 25 mM Mes (pH 7.0). Reaction time for ATPase assay did not exceed 30 s. For each condition, the ATPase assay was done in the presence of 1 mM ouabain and without ouabain. The indicated activities refer to ouabain-sensitive activities which in all cases were more than 95% of the total

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ATPase activities. Beef heart sarcolemmal vesicles were prepared and loaded with 160 mM KCl and 20 mM Mops (pH 7.2) as described before [10]. The sodium pump activity of the sealed inside-out fraction of these vesicles was assayed at 37 °C by measuring the initial rate of ATP-dependent $^{22}Na^+$-uptake by the vesicles [10]. The K^+-loaded vesicles were added to media containing 5 mM $^{22}NaCl$, 140 mM choline chloride, 1 mM $MgCl_2$, 0.2 mM EGTA, 20 mM Mops (pH 7.2), and the indicated concentrations of fatty acid derivatives. Reaction time was 30 s. Under each condition the assay was done in the presence of ATP and without ATP. Inside-out vesicles of human red cell membranes were prepared and assayed for sodium pump activity as described before [13,14]. Vesicles were loaded with 25 mM KCl, 1 mM $MgCl_2$, 0.1 mM EGTA, and 2.5 mM Tris-glycylglycine (pH 6.8); and were added to media containing 4 mM $^{22}NaCl$, 16 mM KCl, 5 mM choline chloride, 1 mM $MgCl_2$, 2.5 mM Tris-glycylglycine (pH 6.8), and the indicated concentrations of the fatty acid derivatives. Under each condition $^{22}Na^+$ uptake by the vesicles was measured after 4 min of incubation at 37 °C, in the presence of ATP and without ATP. It was established that during this period ATP-dependent uptake was a linear function of time. Each data point shown in figures and the table represents the mean of at least three separate determinations. The individual values did not differ from the mean by more than 10%. [γ-^{32}P]ATP and $^{22}Na^+$ were obtained from DuPont-New England Nuclear. 'Vanadate-free' ATP, *D-myo*-inositol 1,4,5-trisphosphate, and fatty acid derivatives were purchased from Sigma or from Serdary Research Laboratories, London, Ontario.

Our previous studies on the purified preparations of Na^+/K^+-ATPase showed that CoA esters of long-chain fatty acids lowered the apparent K_m value of ATP without affecting the maximal velocity; hence causing the stimulation of enzyme activity at suboptimal, but not optimal, ATP concentrations [9–11]. To test for similar effects of diacylglycerols, in experiments of Fig. 1 we compared the effects of palmitoyl-CoA with those of oleic acid and several glycerol esters of oleic acid on the activity of the purified kidney enzyme at a suboptimal concentration of ATP (50 μM). Diolein had no effect on the activity at the concentrations used. 1-Monoolein and 2-monoolein, however, had similar stimulatory effects. The maximal increase obtained with both monoacylglycerols was greater than that noted with the acyl-CoA. In agreement with previous observations on other unsaturated fatty acids [9], oleic acid had a weak activating effect at lower concentrations, and an inhibitory effect at higher concentrations (Fig. 1). When experiments similar to those of Fig. 1 were done at a near-optimal concentration of ATP (2 mM), the monooleins had no significant effects on enzyme activity (data not shown) as expected of activators that

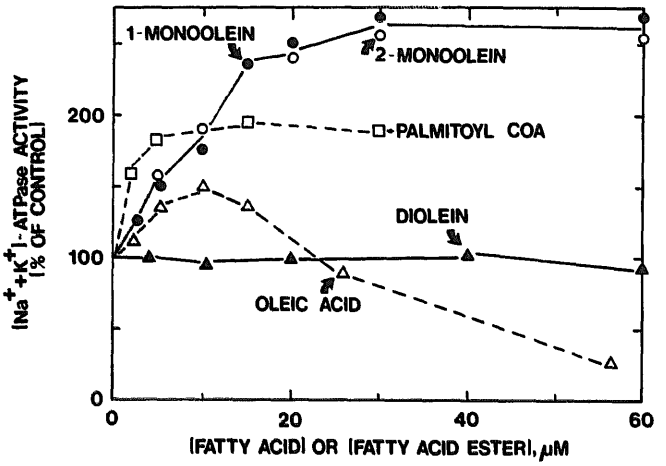

Fig. 1. Effects of 1-monooleoylglycerol, 2-monooleoylglycerol, 1,2-dioleoylglycerol, palmitoyl-CoA, and oleic acid on Na^+/K^+-ATPase activity at a suboptimal concentration (50 μM) of ATP. Assays were done as indicated in the text using a purified preparation of the kidney enzyme. Control activity at 50 μM ATP was 340 μmol of ATP hydrolyzed/mg per h.

lower the enzyme's apparent K_m for substrate without affecting the maximal velocity.

Experiments the results of which are summarized in Table I were done to examine the relation between the structures of acylglycerols and their stimulatory effects on enzyme activity at suboptimal ATP. Only monoacylglycerols stimulated the activity. The tested monoacyl-

TABLE I

Effects of acylglycerols and related compounds on Na^+/K^+-ATPase activity at a suboptimal concentration (50 μM) of ATP

Assays were done as indicated in the text using the purified kidney enzyme. Each indicated compound was used at a concentration range of 1–500 μM, unless indicated otherwise. For each compound maximal activity relative to control, and the concentration at which half-maximal stimulation was obtained (EC_{50}) are presented.

Compound	Maximal activity (% of control)	EC_{50} (μM)
1-Monocapryloyl-*rac*-glycerol	100	–
1-Monodecanoyl-*rac*-glycerol	160	95
1-Monolauroyl-*rac*-glycerol	330	60
1-Monomyristoyl-*rac*-glycerol	195	30
1-Monopalmitoyl-*rac*-glycerol	100	–
1-Monooleoyl-*rac*-glycerol	270	10
1-Monolinoleoyl-*rac*-glycerol	275	10
1-Monoarachidonoyl-*rac*-glycerol	250	5
1,2-Dioleolyl-*rac*-glycerol	100	–
1,2-Dioleolyl-*syn*-glycerol	100	–
1,2-Dilauroyl-*rac*-glycerol	100	–
1,2-Dilauroyl-3-myristoyl-*rac*-glycerol	100	–
L-α-Phosphatidic acid (egg yolk lecithin)	100	–
Dipalmitoyl L-α-phosphatidic acid	100	–
Glycerol	100	–
D-*myo*-Inositol 1,4,5-trisphosphate (0.2–10 μM)	100	–

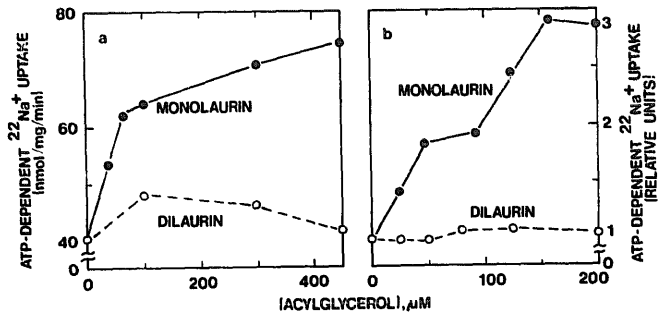

Fig. 2. Effects of 1-monolauroylglycerol and 1,2-dilauroylglycerol on ATP-dependent $^{22}Na^+$ uptake at suboptimal ATP concentrations by: (a) Inside-out beef heart sarcolemmal vesicles; (b) inside-out vesicles of human red cell plasma membranes. Assays were done as indicated in the text. ATP concentration was 100 μM in experiments with sarcolemmal vesicles, and 50 μM in those with red cell vesicles.

glycerols containing unsaturated fatty acids had similar effects. Of the tested monoacylglycerols containing saturated fatty acid, the greatest stimulatory effect (more than 3-fold increase) was obtained with monolaurin. The concentration at which half-maximal activating effect was obtained, however, seemed to decrease with increasing chain length of the fatty acid (Table I). Under the same conditions that monoacylglycerols increased enzyme activity, glycerol, phosphatidic acid, and inositol 1,4,5-trisphosphate had no effects (Table I). In experiments the results of which are not shown, several di- and triacylglycerols other than those used in experiments of Table I were also found to be without effect on enzyme activity at optimal and suboptimal ATP concentrations.

A change in the hydrolytic activity of Na^+/K^+-ATPase need not be accompanied by a similar change in its ion transport activity [15]. Therefore, experiments of Fig. 2 were done to examine the effects of monoacylglycerols on the transport activity of the enzyme in sealed inside-out vesicles of beef heart sarcolemma and human red cell membranes. Because of the pronounced activating effect of monolaurin on the hydrolytic activity (Table I), this compound was used in the transport experiments. In both types of vesicles, monolaurin produced activation of ATP-dependent $^{22}Na^+$ uptake when suboptimal ATP (50–100 μM) was used (Fig. 2). With 2 mM ATP, which is optimal for stimulation of Na^+ uptake in both vesicle systems [10,13,14], monolaurin caused no significant stimulation (data not shown). At the concentrations used in experiments of Fig. 2, monolaurin also had no effect on the passive (i.e., ATP-independent) uptake of $^{22}Na^+$ by the vesicles (data not shown). Dilaurin had no effect on the ATP-dependent uptake in the red cell vesicles (Fig. 2b), but it caused a small stimulatory effect in the cardiac sarcolemmal vesicles (Fig. 2a). No attempt was made to determine if this effect of dilaurin was due to its conversion to monolaurin by the membrane-bound diacylglycerol lipase which is known to exist in myocytes [16].

The second messenger role of diacylglycerols resulting from the agonist-stimulated hydrolysis of phosphatidylinositol is well established. There is also ample evidence to suggest the physiological significance of increased levels of diacylglycerols resulting from receptor-linked breakdown of phosphatidylcholine [17]. The major pathways for the metabolism of a diacylglycerol involve its conversion to phosphatidic acid and its hydrolysis to 2-monoacylglycerol [18]. In some tissues the latter pathway seems to be the predominant one [16]. Where the levels of diacylglycerol and monoacylglycerol upon receptor activation have been measured simultaneously, the magnitude and the time-course of the transient increases in both levels have been found to be about the same [19]. Thus, it is reasonable to consider the possibility that under certain conditions monoacylglycerols may also act as second messengers for the control of some cellular functions. The findings presented here suggest that sodium pump activity is one such function whose well established receptor-linked regulation [1–7] may be mediated through monoacylglycerols.

If monoacylglycerols are indeed second messengers, it is evident that they can cause the activation of the sodium pump rate only when the pump is not saturated with ATP. This raises the important question of whether or not the pump is saturated with ATP under physiological conditions. It is often assumed that normal intracellular concentrations of ATP (say about 5 mM) are optimal for the operation of the pump. This is based on the simple consideration that this ATP level is about 10-fold higher than the apparent K_m of 0.3–0.8 mM for Na^+/K^+-ATPase activity. This K_m value is determined with Na^+ and K^+ concentrations that are optimal for the activity of the isolated enzyme [20]. The normal intracellular concentrations of these ions (high K^+ and low Na^+) are not optimal for pump activity, however, and under these conditions the apparent K_m for ATP is expected to be higher than the above-indicated values [10]. In fact, studies on intact renal tubules, where the ATP dependence of the pump has been thoroughly examined, have demonstrated that the pump is not saturated with ATP [21]. We conclude, therefore, that receptor-linked activation of the sodium pump rate due to a rise in monoacylglycerol levels is a distinct possibility under physiological conditions.

This work was supported by NIH grant HL-36573 awarded by National Heart, Lung and Blood Institute, U.S. Public Health Service/DHHS.

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