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Activation of Na^+/K^+-ATPase by fatty acids, acylglycerols, and related amphiphiles: structure–activity relationship

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Abstract

A number of fatty acids and derivatives have been shown to activate Na^+/K^+-ATPase when ATP is suboptimal. To explore the relation of the structures of these amphiphiles to enzyme activation, the effects of varying amphiphile concentrations on the activity of the highly purified kidney Na^+/K^+-ATPase at 50 μM ATP were determined. Among fatty acids, efficacy (maximal level of activation) and potency were found to be dependent, in different ways, on chain length and unsaturation. Compared to fatty acids, the corresponding alcohols had lower efficacies. Methyl esters of fatty acids inhibited, but CoA esters and monoacyl esters of glycerol activated the enzyme. Relation between chain length and potency among CoA esters and monoacylglycerols was the same as that observed with acids. Diacylglycerols did not activate, but they antagonized the effects of the activator amphiphiles. The substantial specificities of the amphiphile effects support the hypothesis that these ligands bind to a distinct amphipathic peptide segment of the intracellular central loop of the α-subunit to regulate ATP binding to the enzyme. The findings also suggest that direct effects of the changing intracellular levels of fatty acids and derivatives on Na^+/K^+-ATPase should be considered as a possible mechanism for the regulation of its function in the intact cell.

Keywords: ATPase, Na^+/K^+-; Diacylglycerol; Fatty acid; Monoacylglycerol; Sodium pump

1. Introduction

Na^+/K^+-ATPase (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. Inhibitory effects of long chain fatty acids and their derivatives on Na^+/K^+-ATPase were reported long ago [1], and have been subjects of several subsequent studies because of the suspected physiological or pathological significance of such effects [2–6]. In the course of our studies on the interactions of hydrophobic ligands with purified Na^+/K^+-ATPase we noted a second effect of some fatty acids and their CoA esters: at concentrations below those that are inhibitory to the enzyme, these compounds lowered the apparent K_m for ATP; and therefore, activated the enzyme when ATP was suboptimal [7,8]. Subsequently, we explored the mechanism of this reversible activation [9], demonstrated that the ion movements catalyzed by Na^+/K^+-ATPase are also activated by fatty acids and their CoA esters [10,11], and showed that monoacylglycerols, but not diacylglycerols, also activate the transport and hydrolytic functions of the enzyme when ATP is not saturating [12]. Taken together, these findings raise the possibility that some fatty acids and lipid metabolites may regulate the sodium pump when ATP is in short supply, and when the regulator is transiently increased (e.g., through receptor-mediated release of acylglycerols and fatty acids). The aim of the present study was to characterize further the structural requirements for the activation of Na^+/K^+-ATPase by fatty acids and related amphiphiles.

2. Methods

Purified Na^+/K^+-ATPase, with the specific activity of 1000–1500 μmol ATP hydrolyzed/mg protein/h, was prepared from canine kidney medulla, and assayed at 37°C through the determination of the initial rate of release of Pi from [γ-^{32}P]ATP [13]. Reaction mixtures contained 100 mM NaCl, 25 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 50

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μM ATP, 0.2–0.5 μg of enzyme/ml, the indicated concentrations of fatty acid derivatives, 25 mM Tris, and 25 mM Mes (pH 7.0). The ATP concentration used is known to be suboptimal under the assay conditions used [7,8]. Reaction time for the assay was 30 s in order to avoid or minimize the time-dependent irreversible inhibitory effects of the fatty acid derivatives [6]. The assay under each condition was done in the presence of 1 mM ouabain and without ouabain in order to determine the ouabain-sensitive activity. In all cases, this was more than 95% of total ATPase activity. Fatty acids and derivatives were added to reaction media either as solutions or as sonicated suspensions. [γ-^{32}P]ATP was obtained from DuPont New England Nuclear. Ouabain and 'vanadate-free' ATP were purchased from Sigma. Fatty acids and derivatives were obtained from Sigma, Serdary Research (London, Ontario), and Nu Check Prep. (Elysian, MN).

3. Results

3.1. Comparison of fatty acid effects with those of fatty acid derivatives

We have already reported that some fatty acids, fatty acyl CoA esters, and acylglycerols activate the enzyme when ATP is suboptimal [8,9,12]. To compare the activating effects of these classes of compounds quantitatively, in experiments of Fig. 1 the effects of varying concentrations of lauric acid, lauroyl CoA, and mono- and dilauroylglycerols on enzyme activity at 50 μM ATP were determined. Also included in the experiments were the effects of the methyl ester of lauric acid and lauroyl alcohol on enzyme activity. The results showed that at the tested concentrations (a) the acid and the alcohol had biphasic effects; i.e., activation at lower concentrations followed by decline in activating effect and inhibition at higher concentrations; (b) the monoacylglycerol had the highest efficacy, i.e., it produced the highest level of activation; (c) the CoA ester was the most potent activator; i.e., it had the lowest EC_{50}

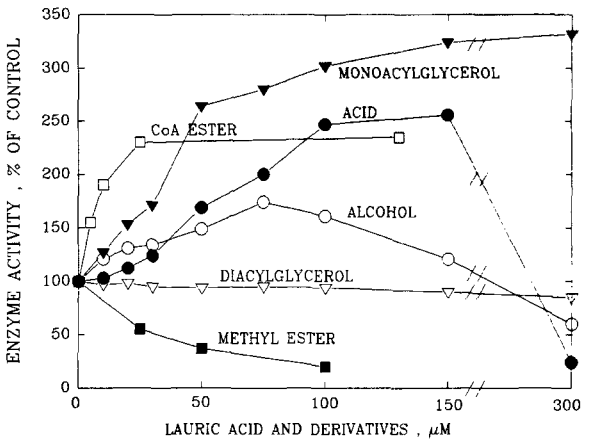

Fig. 1. Effects of varying concentrations of lauric acid and derivatives on Na^+/K^+-ATPase activity at 50 μM ATP.

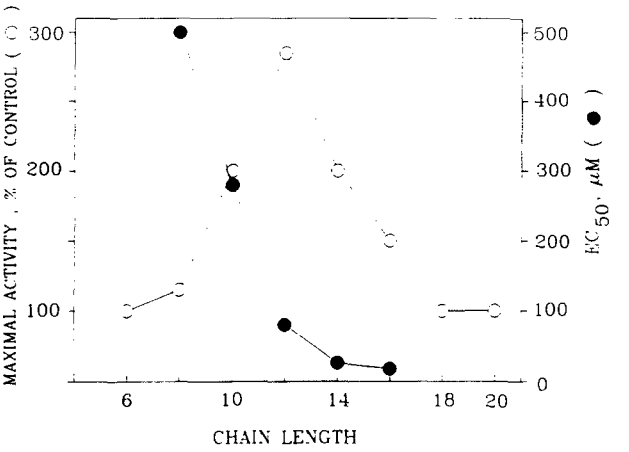

Fig. 2. Effects of chain length on efficacies and potencies of saturated fatty acids as activators of Na^+/K^+-ATPase.

(the concentration producing half-maximal activation); (d) the diacylglycerol was ineffective; and (e) the methyl ester was purely inhibitory. Although a complete set of concentration-effect curves similar to those of Fig. 1 were not obtained for the derivatives of other fatty acids, selected experiments showed that for each saturated long-chain fatty acid (C14-C20), the relative efficacies and potencies of the activating derivatives were about the same as those shown in Fig. 1 for lauric acid derivatives. All methyl esters (C10-C18) were purely inhibitory. All tested diacylglycerols (see below) had no significant activating or inhibitory effects.

3.2. Relation of chain length to activating effect

In experiments the results of which are summarized in Fig. 2, concentration-effect curves (up to 300 μM) were determined for eight saturated fatty acids with different chain lengths. The data showed that when the chain length was below 8 or above 16, there was little or no activating effect. The highest efficacy was that of lauric acid (C12). Among the acids that activated (C8-C16), however, potency increased with increasing chain length (Fig. 2).

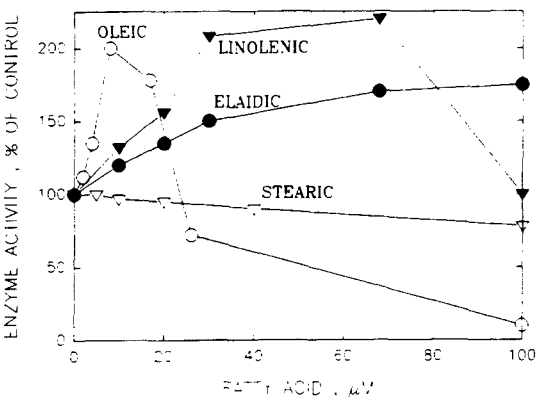

Fig. 3. Effects of unsaturation on the activating effects of fatty acids on Na^+/K^+-ATPase.

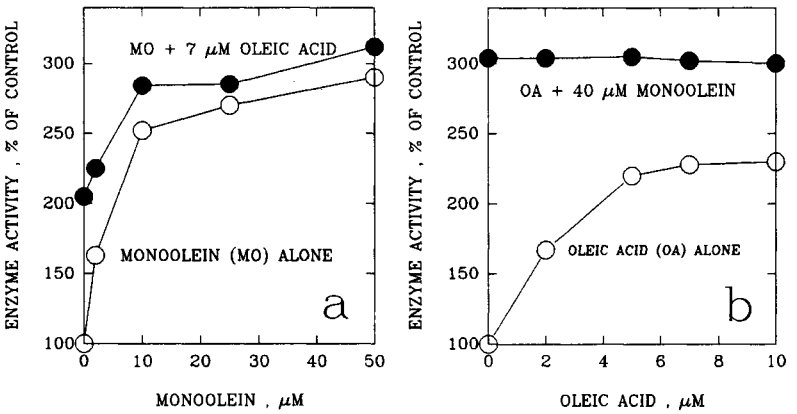

Fig. 4. Combined effects of two activators on Na^+/K^+-ATPase.

The same relation between chain length, efficacy, and EC_{50} obtained in Fig. 2 was observed previously for monoacylglycerols of saturated fatty acids (Table 1 of Ref. [12]). Experiments similar to those of Fig. 2 were also done with several CoA esters of saturated fatty acids, and several saturated fatty alcohols. The pattern of increased potency with increase in chain length was evident within each class, but there was no clear relation between chain length and efficacy (data not shown).

3.3. Effects of unsaturation

As exemplified by the experiments of Fig. 3 with stearic, oleic and linolenic acids, increasing degree of *cis*-unsaturation within the same chain length increased the level of activation produced, but shifted the biphasic concentration-effect curves of the activators to the right.

Experiments the results of which are not presented showed that shifting the position of the *cis* double bond had no significant effect; e.g., the concentration-effect curves for petroselinic acid (18:1, *cis*-6) and vaccenic acid (18:1, *cis*-11) were nearly identical to that of oleic acid (18:1, *cis*-9) shown in Fig. 3.

The geometric isomers of the unsaturated acids exhibited different effects as exemplified by the comparison of the effects of oleic acid (18:1, *cis*-9) with the effects of elaidic acid (18:1, *trans*-9) as shown in Fig. 3. While the latter was less potent than the former, elaidic acid showed only activating effects within the tested range. A similar relationship was observed (data not shown) with other pairs of *cis-trans* isomers: palmitoleic, palmitolaidic; petroselinic, petroselaidic; and vaccinic, transvaccinic. That both isomers activate Na^+/K^+-ATPase is in sharp contrast to the effectiveness of *cis*, but not *trans*, isomers of unsaturated fatty acids on protein kinase C [14,15].

With the same degree of unsaturation, increasing chain length increased the potency of unsaturated acids in the same manner as shown for saturated fatty acids in Fig. 2. Experiments with a number of monoacylglycerols and CoA esters containing long-chain unsaturated acids (C14-C22) showed activating effects of all similar to those shown in Fig. 1, but with no apparent relationship between

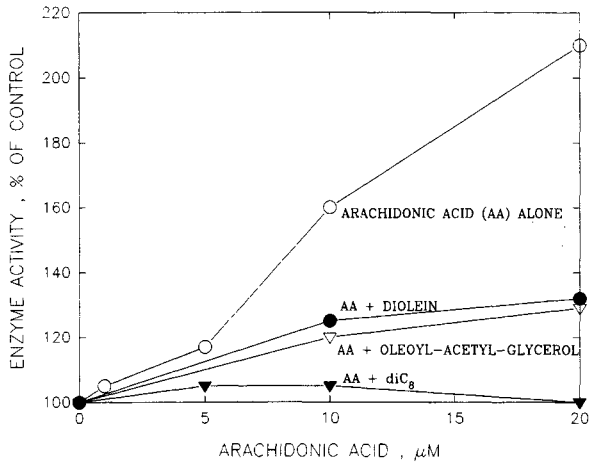

Fig. 5. Antagonism of the activating effects of arachidonic acid on Na^+/K^+-ATPase by diacylglycerols. Each indicated diacylglycerol was used at 50 μM concentration.

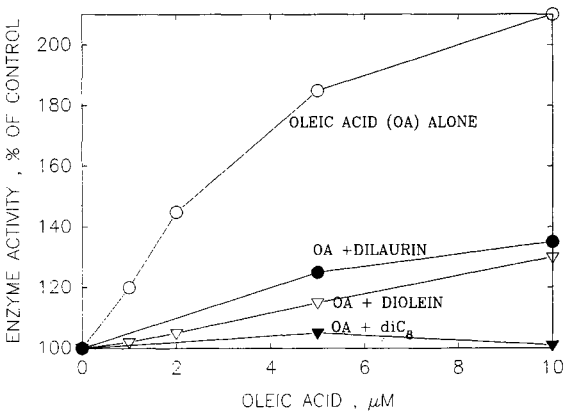

Fig. 6. Antagonism of the activating effect of oleic acid on Na^+/K^+-ATPase by diacylglycerols. Each indicated diacylglycerol was used at 50 μM concentration.

the effect and the number or the location of the unsaturated bonds (see also Refs. [8] and [12]).

3.4. Combined effects of the activators

Whether or not additive effects of two activators were obtained depended on the relative potencies and efficacies of the two activators and the concentrations used. As evident from the examples shown in Fig. 4, the maximal activation produced by the simultaneous presence of two activators did not exceed the maximal effect that could be obtained with the activator having the higher efficacy.

3.5. Antagonism of the activating effects by diacylglycerols

Although diacylglycerols showed no significant activating or inhibitory effects on the enzyme, because of their well established physiological roles, their effects in the presence of related activators were examined. The activating effects of fatty acids were blocked by diacylglycerols as exemplified by the effects of dilaurin, diolein, 1-oleoyl-2-acetylglycerol, and 1,2-dioctanoylglycerol (diC_8) on activation produced by arachidonic acid (Fig. 5) and oleic acid (Fig. 6). Activating effects of monoacylglycerols were also antagonized by diacylglycerols. Of the tested diacylglycerols listed above, diC_8 was the most effective. Its antagonistic effect on activation produced by monolaurin (the most effective monoacylglycerol) is shown in Fig. 7.

4. Discussion

The highly purified Na^+/K^+-ATPase preparation used here contains no vesicles and consists of small membrane fragments that are trilamellar along their length [7,16]. The activating effects of the amphiphiles studied here are, therefore, distinct from their unmasking of latent enzyme activities of crude vesicular preparations. This point was established experimentally before [7].

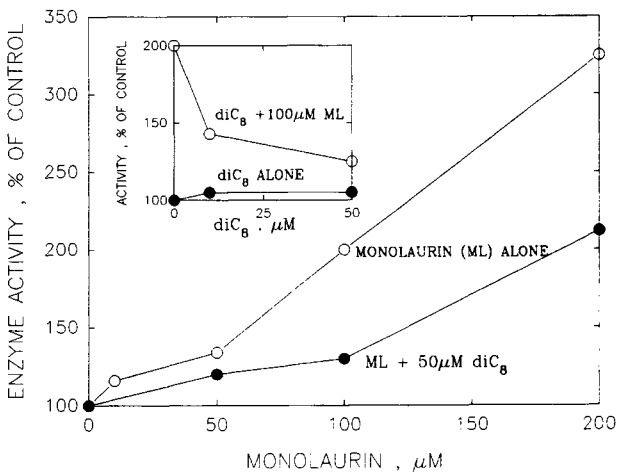

Fig. 7. Antagonism of the activating effect of monolaurin on Na^+/K^+-ATPase by 1,2-dioctanoylglycerol (diC_8).

Although our previous studies with long chain fatty acids and their derivatives showed that a large number of amphiphiles have similar activating effects on the hydrolytic and transport functions of Na^+/K^+-ATPase at suboptimal ATP concentrations [7–11], the present results clearly indicate that there is substantial specificity to the interactions of these ligands with the enzyme. The importance of both hydrophobic and hydrophilic moieties in the activation process is most readily evident from the comparison of the fatty acid effect with those of the corresponding alcohol and methyl ester of the acid (Fig. 1). While the alcohol is a less effective activator than the carboxylate ion, the ester has only inhibitory effects. The activating effects of the CoA ester and the monoacylglycerol (Fig. 1) indicate, however, that there is no rigid requirement for the carboxylate ion, and that the hydrophilicity introduced by the CoA or the hydroxyl groups of the monoacylglycerol is sufficient for the activating effect. The need for the appropriate balance between hydrophilic and hydrophobic moieties, or the appropriate spatial relation of the two, is dramatically emphasized by the fact that esterification of the second hydroxyl group of the monoacylglycerol abolishes its activating effect, and imparts an antagonistic property to the diacylglycerol (Figs. 1, 5–7).

An important issue to be addressed is the potential physiological significance of the activation of Na^+/K^+-ATPase by amphiphiles. Since these effects are observed when ATP is suboptimal, they may seem to be irrelevant to a cell that contains physiological levels of ATP. As we [10] and others [17] have indicated, however, there is evidence to suggest that normal intracellular concentrations of ATP may not be optimal for the function of Na^+/K^+-ATPase in the intact cell. Hence, fluctuating levels of intracellular amphiphiles may affect the transport function of the enzyme under both normal and diminished levels of ATP. The possibility of the compensatory activation of the enzyme by rising intracellular concentrations of fatty acids and their CoA esters in the face of ATP shortage associated with ischemia has been discussed before [10]. There is long-standing evidence [18–20] to indicate that in ischemic tissues with diminished ATP, the levels of free fatty acids and derivatives are increased significantly.

The other situation under which the possibility of amphiphile-induced regulation of Na^+/K^+-ATPase should be considered, is when there is a receptor-linked change in cellular sodium pump activity, and it is known that activation of the same receptor leads to hydrolysis of membrane phosphatidylinositol phosphates. Such receptor-linked activations or inhibitions of ion movements mediated by Na^+/K^+-ATPase have been noted in several studies [21–25], and in all cases attention has been focused on the resulting activation of protein kinase C by the released diacylglycerols, and phosphorylation of Na^+/K^+-ATPase by protein kinase C. However, although the α-subunit of Na^+/K^+-ATPase is a substrate for protein kinase C, no

definite changes in the properties of Na^+/K^+-ATPase resulting from this phosphorylation have been demonstrated to date [26–29]. This, and our findings presented here suggest that some or all of the noted receptor-linked alterations of Na^+/K^+-ATPase in intact cells may be due to direct effects of released diacylglycerols and their metabolites (monoacylglycerols and free fatty acids) on the enzyme. If there are transient changes in the concentration of these three classes of amphiphiles in the vicinity of the plasma membrane, our results show that whether Na^+/K^+-ATPase activity is altered or not depends on the balance between the activating effects of monoacylglycerols and fatty acids, the antagonism of these effects by diacylglycerols, and inhibitory effects of higher levels of free fatty acids. The complexities of these direct effects, and the possible indirect effects of protein kinase C on Na^+/K^+-ATPase, clearly indicate that it will be difficult to identify the precise causes of the above receptor-linked changes in sodium pump activity.

The demonstration of the highly specific nature of amphiphile effects on Na^+/K^+-ATPase also has implications for studies on the relation of enzyme structure to its function. We showed before [7,9] that the activating effects of amphiphiles on the enzyme occur without a lag, and are readily reversible; suggesting the rapid equilibrium bindings of these ligands to discrete sites of the extramembraneous segments of the enzyme subunits. Subsequently, these sites were localized to the intracellular rather than the extracellular domains of the enzyme [11]. Since the sequences of the enzyme subunits are now known, and since significant progress on the characterization of transmembrane segments of the subunits has been made, we may ask if there is independent evidence in support of the existence of specific binding sites for amphiphilic ligands on the intracellular domains of the subunits. Studies of several laboratories [30–33] have identified a highly hydrophobic segment of the α-subunit on the large central intracellular loop between the 4th and 5th transmembrane helices of this subunit. Modyanov et al. [33] have suggested that this peptide segment (Val^{545}-Arg^{590}) may in fact be bound to the intracellular surface of the plasma membrane bilayer, or be partially imbedded in it; and this suggestion has been further reinforced by the recent experiments of Lutsenko and Kaplan [34]. Since this segment is close to several residues of the central loop that have been implicated in nucleotide binding [35], and since the segment has also been shown to have structural similarities to the ATP binding sites of adenylate kinase and related enzymes [36], it is reasonable to suggest that hydrophobic interactions of this peptide segment with the bilayer may regulate ATP binding to the enzyme. Extending this argument, it also seems reasonable to suggest that the amphiphilic ligands studied here exert their effects on the apparent K_m of ATP through 'competition' with the bilayer for binding to the Val^{545}-Arg^{590} segment of the central loop. It remains to be seen if the nature and specificities of amphiphile effects on enzyme activity, as revealed by the present studies, can be altered by appropriate structural modifications within this segment of the α-subunit.

Acknowledgements

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