Mechanism of the Control of (Na\(^+\) + K\(^+\))-ATPase by Long-chain Acyl Coenzyme A*

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Long-chain fatty acid esters of CoA activate (Na\(^+\) + K\(^+\))-ATPase (the sodium pump) when ATP is suboptimal. To explore the nature of the interactions of these CoA derivatives with the pump, reversible effects of palmitoyl-CoA on the purified membrane-bound kidney enzyme were studied under conditions where interference from the irreversible membrane-damaging effect of the compound was ruled out. With 50 μM ATP, while saturating palmitoyl-CoA increased (Na\(^+\) + K\(^+\))-ATPase activity, it caused partial inhibition of Na\(^+\)-ATPase activity without affecting the steady-state level of the phosphoenzyme. Palmitoyl-CoA did not change the K\(_{a5}\) of ATP for Na\(^+\)-ATPase, but it altered the complex Na\(^+\) activation curve to suggest the antagonism of the low-affinity, but not the high-affinity, Na\(^+\) sites. At a low ATP concentration (0.5 μM), K\(^+\) inhibited Na\(^+\)-ATPase as expected. In the presence of palmitoyl-CoA and 0.5 μM ATP, however, K\(^+\) became an activator, as it is at high ATP concentrations. The activating effect of palmitoyl-CoA on (Na\(^+\) + K\(^+\))-ATPase activity was reduced with increasing pH (6.5–8.5), but its inhibitory effect on Na\(^+\)-ATPase was not altered in this pH range. The data show two distinct actions of palmitoyl-CoA: 1) blockade of the extracellular "allosteric" Na\(^+\) sites whose exact role in the control of the pump is yet to be determined, and 2) activation of the pump through increased rate of K\(^+\) deocclusion. Since in their latter action the fatty acid esters of CoA are far more effective than ATP at a low-affinity regulatory site, we suggest that these CoA derivatives may be the physiological ligands of this regulatory site of the pump.

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The mechanism of the control of (Na\(^+\) + K\(^+\))-ATPase by long-chain acyl Coenzyme A is explored. The study shows that palmitoyl-CoA has both activating and inhibitory effects on the ATPase activity, depending on the pH and the concentration of ATP. The mechanism involves the interaction of CoA derivatives with the extracellular sites of the sodium pump, suggesting that these CoA esters may play a physiological role in the control of the sodium pump. The results are important for understanding the physiological control of the sodium pump and the potential role of long-chain acyl CoA derivatives in cellular processes.
Control of (Na\(^+\) + K\(^+\))-ATPase by Fatty Acyl-CoA

Our previous experiments showing that CoA esters of fatty acids decreased K\(_{0.5}\) of ATP for (Na\(^+\) + K\(^+\))-dependent ATPase activity suggested that these compounds mimic the effect of ATP at a low-affinity ATP site and are not mutually exclusive. Since there have been disagreements on whether or not the high- and low-affinity ATP sites are the same (6, 7) and since only a high-affinity ATP site is apparent in the expression of the Na\(^+\)-dependent ATPase activity of the enzyme, it became of interest to examine the acyl-CoA effects on this activity. In the experiments of Fig. 2, the effects of varying concentrations of palmitoyl-CoA on Na\(^+\)-dependent and (Na\(^+\) + K\(^+\))-dependent activities at the same substrate concentration (50 \(\mu\)M) were compared. In agreement with previous results (1), (Na\(^+\) + K\(^+\))-dependent activity increased more than 2-fold. The Na\(^+\)-dependent activity, however, was inhibited by palmitoyl-CoA to the maximal extent of 60–70%. Of particular interest was this partial nature of the inhibition (Fig. 2), suggesting that ATP and palmitoyl-CoA are not mutually exclusive.

Reversibility of Palmitoyl-CoA Inhibition of Na\(^+\)-dependent ATPase Activity—Although due to the choice of assay conditions, the inhibition of Na\(^+\)-dependent activity by palmitoyl-CoA (Fig. 2) was not likely to be due to the irreversible membrane-damaging effect of the compound (see above), it was important to establish this point unambiguously. In the experiments of Fig. 3, the enzyme that was placed in the medium for the assay of Na\(^+\)-dependent activity was inhibited to the extent of about 60% by the simultaneous addition of palmitoyl-CoA. After 60 s, K\(^+\) was added to the medium, and (Na\(^+\) + K\(^+\))-dependent activity was assayed for 20 s. This activity was more than 90% of the same activity of a control enzyme sample that had not been preincubated with palmitoyl-CoA (Fig. 3). Had the initial 60% inhibition of the Na\(^+\)-dependent activity been due to irreversible inactivation, the subsequent (Na\(^+\) + K\(^+\))-dependent activity could not have been more than 40% of the control. These data clearly indicate the reversibility of the inhibition of the Na\(^+\)-dependent activity.

Relation of Palmitoyl-CoA Site to ATP and Na\(^+\) Sites of Na\(^+\)-dependent ATPase Activity—Experiments, the results of which are not presented, showed that the K\(_{0.5}\) value of ATP for Na\(^+\)-dependent ATPase activity (0.2 \(\mu\)M) was not changed significantly in the presence of inhibitory concentrations of palmitoyl-CoA. Also, a 100-fold change in the concentration of ATP had little effect on the K\(_{0.5}\) of palmitoyl-CoA as an activator.

Comparison of Palmitoyl-CoA Effects on (Na\(^+\) + K\(^+\))- and Na\(^+\)-dependent ATPase Activities—Our previous experiments showing that CoA esters of fatty acids decreased K\(_{0.5}\) of ATP for (Na\(^+\) + K\(^+\))-dependent ATPase activity suggested that these compounds mimic the effect of ATP at a low-affinity ATP site and are not mutually exclusive. Since there have been disagreements on whether or not the high- and low-affinity ATP sites are the same (6, 7) and since only a high-affinity ATP site is apparent in the expression of the Na\(^+\)-dependent ATPase activity of the enzyme, it became of interest to examine the acyl-CoA effects on this activity. In the experiments of Fig. 2, the effects of varying concentrations of palmitoyl-CoA on Na\(^+\)-dependent and (Na\(^+\) + K\(^+\))-dependent activities at the same substrate concentration (50 \(\mu\)M) were compared. In agreement with previous results (1), (Na\(^+\) + K\(^+\))-dependent activity increased more than 2-fold. The Na\(^+\)-dependent activity, however, was inhibited by palmitoyl-CoA to the maximal extent of 60–70%. Of particular interest was this partial nature of the inhibition (Fig. 2), suggesting that ATP and palmitoyl-CoA are not mutually exclusive.

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FIG. 3. Reversibility of palmitoyl-CoA inhibition of Na\(^+\)-dependent ATPase. ATP concentration was 50 \(\mu\)M. Enzyme concentration was 5 \(\mu\)g/ml. After the indicated periods, enzyme samples were assayed for Na\(^+\)-dependent activity in the absence (○) and presence (●) of 30 \(\mu\)M palmitoyl-CoA. After 60 s, 20 mM K\(^+\) was added to one set of tubes containing the latter reaction mixture, and the enzyme was assayed 20 s later (●). Other samples were assayed for (Na\(^+\) + K\(^+\))-dependent activity in the absence (○) and presence (□) of 30 \(\mu\)M palmitoyl-CoA. Other conditions are as indicated under “Experimental Procedures.”

FIG. 4. Effects of varying concentrations of Na\(^+\) on Na\(^+\)-dependent ATPase activity in the absence (○) and presence (●) of 5 \(\mu\)M palmitoyl-CoA. Other conditions are as indicated under “Experimental Procedures.”

Inhibitor of Na\(^+\)-dependent activity (Fig. 2). When the effect of 30 \(\mu\)M palmitoyl-CoA on the steady-state level of acid-stable phosphoenzyme obtained from 50 \(\mu\)M ATP in the presence of 100 mM Na\(^+\) and 5 mM Mg\(^++\) was determined at 24 °C in four separate experiments, the control level was 2.1 ± 0.2 nmol of P\(_i\)/mg of protein and that in the presence of palmitoyl-CoA was 2.3 ± 0.2 nmol of P\(_i\)/mg of protein. These data, in conjunction with the partial nature of the inhibition of Na\(^+\)-dependent ATPase caused by palmitoyl-CoA (Fig. 2), clearly show that palmitoyl-CoA and ATP (at the high-affinity catalytic site) may bind simultaneously and without significant interactions.

The experiments of Fig. 4 showed a dramatic effect of palmitoyl-CoA on the Na\(^+\) activation curve of the Na\(^+\)-dependent ATPase activity. The curve for the control enzyme had an intermediate plateau in the range of 5–10 mM Na\(^+\), as has been noted repeatedly (8). Palmitoyl-CoA had no significant effect on activity at Na\(^+\) concentrations below this plateau, but it inhibited at Na\(^+\) concentrations above the plateau (Fig. 4).

Effect of Palmitoyl-CoA on K\(^+\) Inhibition of Na\(^+\)-dependent ATPase Activity—When ATP concentration is considerably below the \(K_{\text{m}}\) value for the low-affinity ATP site, K\(^+\) inhibits Na\(^+\)-dependent ATPase activity because the release of K\(^+\) from the enzyme is too slow without ATP being at the low-affinity site (7). The experiments of Fig. 5 showed that palmitoyl-CoA overcame such an inhibitory effect of K\(^+\) completely, providing further evidence for an effect of the CoA ester similar to that of ATP at its low-affinity site.

Effects of Palmitoyl-CoA at Different pH Values—The rate of release of K\(^+\) from the enzyme (or the rate of deocclusion of K\(^+\)) is increased not only by ATP at the low-affinity site, but also by an increase in pH (9, 12). It was of interest, therefore, to see how pH affected the palmitoyl-CoA activation of (Na\(^+\) + K\(^+\))-dependent activity at suboptimal ATP concentrations. Experiments of Fig. 6, in which this activity was measured at 50 \(\mu\)M ATP, showed that (a) the activity of the control enzyme increased with pH in the range of 6.5–8.5; (b) whereas palmitoyl-CoA increased activity at all pH values, its effect was greater at lower pH values; and (c) in the presence of saturating palmitoyl-CoA, an increase in pH had no activating effect. Evidently, palmitoyl-CoA, ATP at the low affinity site, and deprotonation of the enzyme have similar effects on the rate of K\(^+\) deocclusion. The maximal inhibitory effect of a saturating palmitoyl-CoA concentration on Na\(^+\)-dependent ATPase activity (about 60% inhibition) was not significantly affected by a change of pH within the above range (data not shown).
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**DISCUSSION**

These results (Figs. 5 and 6) provide further support for our previous suggestion (1, 2) that the activating effects of a fatty acyl-CoA on (Na\(^{+} + K\(^{+}\))\text{-dependent ATPase and ATP-dependent Na\(^{+}/K\(^{+}\)} exchange are due to its ATP\text{-like effect on the rate of release of K\(^{+}\) from the K\(^{+}\)-occluded conformation of the enzyme within the Albers-Post cycle. Because this effect of the CoA derivative is observed in the presence of low concentrations of ATP, on the basis of our experiments alone, we cannot say whether the acyl-CoA is binding to the low-affinity site whose effects are blocked by the pump beyond those on K\(^{+}\) deocclusion. The nature of these effects, however, is less clear. There is ample evidence (8) to indicate that the complex Na\(^{+}\) activation curve of the Na\(^{+}\)-dependent ATPase of the purified enzyme involves multiple intracellular and extracellular Na\(^{+}\) sites of the pump and that the low-affinity Na\(^{+}\) sites whose effects are blocked by palmitoyl-CoA (Fig. 4) are of extracellular nature. It is not clear, however, if these low-affinity Na\(^{+}\) sites are purely

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