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THE INFLUENCE OF ADENOSINE 3′,5′-MONOPHOSPHATE UPON THE ACTIVITY OF THE MEMBRANE-ASSOCIATED (Ca^{2+}+Mg^{2+})-DEPENDENT ADENOSINE TRIPHOSPHATASE OF THE HUMAN ERYTHROCYTE

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Summary

The phosphohydrolase activity of the membrane-associated (Ca^{2+}+Mg^{2+})-dependent adenosine triphosphatase (ATPase) of the human erythrocyte can be inhibited by micromolar or nanomolar concentrations of cyclic AMP. Millimolar concentrations of cyclic AMP are less effective. The inhibitory effect of cyclic AMP is potentiated in the presence of the phosphodiesterase inhibitor, theophylline.

The concentration of Ca^{2+} within the human erythrocyte is less than micromolar while normal serum contains approx. 2.5 mM Ca^{2+}, half of which is protein-bound. The lower intracellular concentration of Ca^{2+} in the erythrocyte is maintained through the agency of a membrane-associated (Ca^{2+}+Mg^{2+})-dependent ATPase which transports Ca^{2+} from inside to outside the cell concomitantly with the hydrolysis of ATP. Both the phosphohydrolase [1—4] and the Ca^{2+}-transport [5,6] activities of this ATPase have been shown to be promoted by Ca^{2+} in conjunction with a small (16 700 dalton), acidic (p*I*, 3.9) Ca^{2+}-binding protein referred to as the calcium-dependent regulator (or calmodulin). The calcium-dependent regulator and Ca^{2+} have also been shown to promote Ca^{2+} transport implemented by the (Ca^{2+}+Mg^{2+})-dependent ATPase of the cardiac sarcoplasmic reticulum [7] and the synaptosomal membrane [8]. In addition, the calcium-dependent

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Abbreviations used: cyclic AMP, adenosine 3′,5′-monophosphate; cyclic GMP, guanosine 3′,5′-monophosphate; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N,N*′-tetraacetic acid.

TABLE I

EFFECT OF CYCLIC AMP UPON (Ca^{2+}+Mg^{2+})-DEPENDENT PHOSPHOHYDROLASE ACTIVITY OF HUMAN ERYTHROCYTE MEMBRANES

Erythrocyte membranes were isolated in an imidazole solution (I) that was either 257 or 305 mosM, or, alternatively, in a 30 mosM potassium phosphate buffer solution (P). Following isolation, preparations were maintained at 4°C, non-frozen (NF), or frozen (F) at −60°C and maintained frozen at −15°C. (Ca^{2+}+Mg^{2+})-dependent phosphohydrolase activity was routinely determined within 15 h subsequent to the completion of the isolation procedures, unless indicated. Data presented in columns 3 and 4 were obtained using the same preparation, one portion of which had been maintained non-frozen and the other, frozen. Data presented in columns 5, 6, 7, and 8 were again obtained using the same preparation, portions of which had been maintained under different conditions. In each instance, (Ca^{2+}+Mg^{2+})-dependent phosphohydrolase activity is given, followed by the standard error (S.E.). Results are expressed in the following units: nmol (P) $\cdot h^{-1} \cdot mg^{-1}$.

Cyclic AMP concn.	I-257 NF	I-305 NF	I-305 F	P-30 NF	P-30 NF[a]	P-30 F	P-30 NF, F[b]	P-30 F[c]
None	88 ± 3	607 ± 3	514 ± 3	665 ± 5	229 ± 2	578 ± 4	51 ± 2	91 ± 1
1 mM	80 ± 1	602 ± 4	475 ± 3	668 ± 5	230 ± 3	575 ± 3	40 ± 2	91 ± 3
10 μM	88 ± 2	593 ± 3	471 ± 3	671 ± 4	214 ± 2	574 ± 4	37 ± 1	82 ± 3
1 μM	69 ± 1	550 ± 3	477 ± 3	664 ± 4	210 ± 2	579 ± 4	37 ± 2	70 ± 1
10 nM	67 ± 3	591 ± 4	473 ± 4	618 ± 5	207 ± 2	562 ± 4	36 ± 2	59 ± 4
1 nM	69 ± 3	604 ± 3	450 ± 4	652 ± 5	224 ± 2	539 ± 4	39 ± 2	—

[a] Membranes maintained non-frozen for 7 days.
[b] Membranes maintained non-frozen for 7 days, then frozen and maintained frozen for 12 days.
[c] Membranes frozen immediately following completion of isolation procedure and maintained frozen for 44 days.

regulator and Ca^{2+} also modulate the activities of cyclic nucleotide phosphodiesterases [9—11], adenylate cyclase [12], myosin light-chain kinase [13], (cyclic AMP-insensitive) glycogen-synthase kinase [14] and phosphorylase kinase [15]. Since, in the cardiac sarcoplasmic reticulum, Ca^{2+} transport by the (Ca^{2+}+Mg^{2+})-dependent ATPase appears to be modulated not only by Ca^{2+} and the calcium-dependent regulator, but also by cyclic AMP and a membrane-associated, cyclic AMP-dependent protein kinase [16], we undertook studies designed to determine whether or not Ca^{2+} transport in the erythrocyte is also modulated by cyclic AMP and a protein kinase in addition to the enhancement induced by Ca^{2+} and the calcium-dependent regulator. Here, we report that the phosphohydrolase activity of the (Ca^{2+}+Mg^{2+})-dependent ATPase of the human erythrocyte membrane can be inhibited by the addition of micromolar or nanomolar concentrations of cyclic AMP. We suggest that the energy-dependent translocation of Ca^{2+} across the erythrocyte membrane can be modulated by means of a mechanism involving cyclic AMP and a membrane-associated protein kinase.

Human erythrocyte membranes were isolated following cell lysis in the presence of a hypotonic potassium phosphate buffer (pH 7.4) or 257 or 305 mosM imidazole (pH 7.4). Osmolarities were determined using an Advanced digital osmometer. After having been washed three or more times, virtually colorless membranes were obtained. Those obtained in an imidazole solution were then washed additionally with a 40 mM histidine/40 mM imidazole solution (pH 7.4) and maintained in this medium. The phosphohydrolase activities exhibited by these membranes were determined using the following procedure. Media comprising 18 mM histidine/18 mM imidazole buffer (pH 7.1), 3 mM $MgCl_2$, and 100 μl of the membrane preparation (having a protein concentration of 5 to 10 mg/ml) were prepared. When (Ca^{2+}+Mg^{2+})-dependent phosphohydrolase activity was to be determined,

0.1 mM $CaCl_2$ and 0.1 mM ouabain were present. The $(Na^+ + K^+)$-dependent phosphohydrolase activity of these preparations was determined in the presence of 80 mM NaCl, 15 mM KCl and 0.5 mM EGTA with neither $CaCl_2$ nor ouabain added. Mg^{2+}-dependent and other phosphohydrolase activities were determined using media that lacked the $CaCl_2$, NaCl and KCl while containing EGTA and ouabain. The phosphohydrolase reaction was initiated by the addition of Na_2ATP^{2-} (containing $3 \cdot 10^5$ cpm [γ-^{32}P]ATP) in such an amount as to achieve a final concentration of 3 mM. Non-enzymatic hydrolysis of ATP was monitored using media lacking only the membrane preparation. Following incubation at 37°C for 20 min, enzymatic hydrolysis was terminated by the addition of 1.0 ml of 5% (w/v) trichloroacetic acid containing 2 mM KH_2PO_4. Then, 1.0 ml of 1.25 M H_2SO_4 containing 5% (w/v) ammonium molybdate was added, followed by 5 ml of isobutanol/benzene (1:1, v/v). After thorough mixing for 30 s, the solutions were centrifuged in order to promote phase separations and an aliquot of the organic phase was transferred to a vial, 8 ml of Aquasol were added and radioactivity was determined by means of liquid scintillation spectrometry.

The $(Ca^{2+} + Mg^{2+})$-dependent phosphohydrolase activity of human erythrocyte membranes is inhibited in the presence of micromolar or nanomolar concentrations of cyclic AMP. Millimolar concentrations of this cyclic nucleotide are less effective. We have also found that cyclic GMP induces inhibition similar to that seen in the presence of cyclic AMP although the optimum concentration of cyclic GMP is approx. 10 μM (data not shown). Both the profile of cyclic AMP-concentration effect and the relative effectiveness of cyclic AMP and cyclic GMP suggest that the inhibition imposed by the cyclic nucleotides upon $(Ca^{2+} + Mg^{2+})$-dependent phosphohydrolase activity is mediated by the cyclic AMP-dependent protein kinase of the human erythrocyte membrane. This enzyme has been isolated, purified and characterized in our laboratory. The phosphotransferase activity of the membrane-derived protein kinase is enhanced less in the presence of millimolar concentrations of cyclic AMP than it is when micromolar or nanomolar concentrations are added and cyclic GMP appears to be approx. 1% as effective as cyclic AMP with respect to promoting protein phosphorylation [17]. Our studies reported here also demonstrated that (0.1 mM) theophylline can potentiate the inhibitory effect of cyclic AMP, particularly when freshly prepared membrane preparations are being used. We interpret our observations to suggest that the $(Ca^{2+} + Mg^{2+})$-dependent phosphohydrolase activity of the erythrocyte membrane can be modulated as a result of cyclic AMP-induced phosphorylation of a membrane-associated protein. Presumably, protein phosphorylation induces a diminution in phosphohydrolase activity. However, we have at present, not eliminated the possibility that protein phosphorylation can, in fact, induce an enhancement in $(Ca^{2+} + Mg^{2+})$-dependent phosphohydrolase activity in the erythrocyte membrane just as it does in the cardiac sarcoplasmic reticulum [18]. If phosphorylation of the erythrocyte membrane can promote the activities of the $(Ca^{2+} + Mg^{2+})$-dependent ATPase, then the inhibition that we observe here could reflect cyclic AMP-dependent dephosphorylation under the conditions of our experiments. Phosphoprotein phosphatase activity has been shown to be associated with the human erythrocyte membrane [19] although

it has not been established that this activity is enhanced by cyclic AMP. On the other hand, the phosphotransferase reaction catalyzed by a type I, cyclic AMP-dependent protein kinase has been shown to be reversible with dephosphorylation also promoted by cyclic AMP [20]. Therefore, it is envisaged that with additional ADP being generated at the erythrocyte membrane by the $(Ca^{2+}+Mg^{2+})$-dependent phosphohydrolase reaction, dephosphorylation, catalyzed by the membrane-associated cyclic AMP-dependent protein kinase, could be promoted. Finally, as seen in Table I, the inhibitory effect of cyclic AMP is observed when using membrane prepared in either an imidazole solution or a phosphate buffer. Thus, it is implied that the presence of the calcium-dependent regulator is not required in order for the influence of cyclic AMP to be observed, since although membrane preparations obtained in approx. 300 mosM imidazole contain this protein, preparations obtained in hypotonic phosphate buffers do not [21].

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References

1 Farrance, M.L. and Vincenzi, F.F. (1977) Biochim. Biophys. Acta 471, 59—66
2 Gopinath, R.M. and Vincenzi, F.F. (1977) Biochem. Biophys. Res. Commun. 77, 1203—1209
3 Jarrett, H.W. and Penniston, J.T. (1977) Biochem. Biophys. Res. Commun. 77, 1210—1216
4 Jarrett, H.W. and Penniston, J.T. (1978) J. Biol. Chem. 253, 4676—4682
5 Hinds, T.R., Larsen, F.L. and Vincenzi, F.F. (1978) Biochem. Biophys. Res. Commun. 81, 455—461
6 MacIntyre, J.D. and Green, J.W. (1978) Biochim. Biophys. Acta 510, 373—377
7 Katz, S. and Remtulla, M.A. (1978) Biochem. Biophys. Res. Commun. 83, 1373—1379
8 Kuo, C.-H., Ichida, S., Matsuda, T., Kakiuchi, S. and Yoshida, H. (1979) Life Sci. 25, 235—240
9 Cheung, W.Y. (1967) Biochem. Biophys. Res. Commun. 29, 478—482
10 Wolff, D.J. and Brostrom, C.O. (1974) Arch. Biochem. Biophys. 163, 349—358
11 Waisman, D.M., Stevens, F.C. and Wang, J.H. (1978) J. Biol. Chem. 253, 1106—1113
12 Brostrom, C.O., Huang, Y.-C., Brekenridge, B.M. and Wolff, D.J. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 64—68
13 Yazawa, M. and Yagi, K. (1977) J. Biochem. (Tokyo) 82, 287—289
14 Srivastava, A.K., Waisman, D.M., Brostrom, C.O. and Soderling, T.R. (1979) J. Biol. Chem. 254, 583—586
15 Walsh, K.X., Millikin, D.M., Schlender, K.K. and Reimann, E.M. (1979) J. Biol. Chem. 254, 6611—6616
16 Kirchberger, M.A., Tada, M. and Katz, A.M. (1974) J. Biol. Chem. 249, 6166—6173
17 Grant, B.F., Breithaupt, T.B. and Cunningham, E.B. (1979) J. Biol. Chem. 254, 5726—5733
18 Tada, M., Ohmori, F., Yamada, M. and Abe, H. (1979) J. Biol. Chem. 254, 319—326
19 Greenquist, A. and Shohet, S.B. (1973) Blood 42, 997
20 Shizuta, Y., Beavo, J.A., Bechtel, P.J., Hofmann, F. and Krebs, E.G. (1975) J. Biol. Chem. 250, 6891—6896
21 Farrance, M.L. and Vincenzi, F.F. (1977) Biochim. Biophys. Acta 471, 49—58