In Vivo Insulin Effect on ATPase Activities in Erythrocyte Membrane From Insulin-Dependent Diabetics

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SUMMARY

Na^+-K^+–dependent ouabain-sensitive ATPase and Mg^{2+}-ATPase have been assayed in the erythrocyte membranes of control subjects and in uncontrolled type I (insulin-dependent) diabetics. A decrease in Na^+-K^+-ATPase activity was observed in the patients that was significantly correlated with glycemia. The Mg^{2+}-ATPase was increased moderately, and no correlation with glycemia was found. To study the in vivo effect of insulin, ATPase activities were measured in uncontrolled diabetics before and after a 24-h continuous insulin perfusion administered by means of an artificial pancreas. ATPase activities were corrected after normalization of glycemia. It therefore seems that glycemia and/or insulinemia are involved in the regulation of erythrocyte Na^+-K^+ ouabain-sensitive ATPase and to a lesser extent in that of Mg^{2+}-dependent ATPase. *Diabetes* 36:991–95, 1987

The presence of specific insulin receptors at the surface of erythrocyte plasma membrane is well documented (1), but the physiological relevance of this observation is not clear. In target cells the first step in insulin action is the binding of the hormone to receptors, a metabolic event that stimulates receptor phosphorylation (2). To act on membrane or intracellular proteins (3), the insulin receptor is believed to trigger various processes through individual or coordinated routes (4). The effect of insulin or receptor (insulin bound) on membrane-bound enzymes has been reported extensively (5–8), and direct action of the hormone on isolated plasma membranes of liver (9) and erythrocyte (5) has been demonstrated. In erythrocytes the in vitro hormone-receptor interaction could affect membrane fluidity (5), resulting in an alteration of the membrane microenvironment responsible for variations in activity of membrane-bound enzymes [e.g., Na^+-K^+-ATPase (EC 3.6.1.4.); 10].

Na^+-K^+-ATPase inserted into plasma membranes generates and maintains high K^+ and low Na^+ concentrations in the cytoplasm (11). The resulting difference in the steady-state concentration of these cations provides the potential energy to maintain cellular volume, drive uptake of nutriments, and to create the resting potentials of cells. ATPase is an integral membrane protein. Its activity seems to greatly depend on the presence of phosphatidylserine (12) or phosphatidylinositol (13) and on the phospholipid fatty acid composition. Notably, ATPase activity is increased after incorporation of polyunsaturated fatty acids into the membrane lipid components (14,15). Mg^{2+}-ATPase (EC 3.6.1.3.) also closely binds to the membrane; its activity is lower after incubation of erythrocytes with linoleic acid (15) but shows no sensitivity to insulin treatment (5) or membrane fluidity (10), in contrast to Na^+-K^+-ATPase.

We have previously described lipid alterations (16) and decreased fluidity (17) in erythrocyte membranes from insulin-deprived type I diabetics. These observations prompted us to study human erythrocyte membrane–bound Na^+-K^+-ATPase and Mg^{2+}-ATPase activities in uncontrolled type I diabetics before and after insulin administration. Changes in ATPase activity have been reported with different tissues from experimental diabetic rats. ATPase was decreased in nerve (18), heart (7), and glomeruli (19) and increased in renal medulla and cortex (20). Only one study has been achieved in human subjects (21).

MATERIALS AND METHODS

Subjects. Blood was obtained in the fasting state at 0800 h from 14 healthy controls (8 men, 6 women) and 14 uncontrolled type I diabetic patients before insulin injection (12 men, 2 women). The controls were from the laboratory staff, and all diabetics were inpatients at the Department of Dia-

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TABLE 1
ATPase activities in erythrocyte plasma membrane from control men and women

Control subjects	*n*	Age (yr)	ATPase activities (μmol P_i · mg^{-1} protein · min^{-1})		
			Total	Na^+-K^+	Mg^{2+}
Men	8	41 ± 3	1.00 ± 0.10	0.41 ± 0.03	0.59 ± 0.09
Women	6	34 ± 3	1.06 ± 0.11	0.48 ± 0.05*	0.57 ± 0.11

ATPase activities are measured in purified erythrocyte plasma membranes as described in text. Values are means ± SD. Significant differences between women and men were obtained by Student's *t* test.
*$P < .05$.

betology. Informed consent was obtained from all subjects. Ages ranged from 20 to 73 yr (mean ± SD 51 ± 18) for diabetics and from 20 to 54 yr (mean 38 ± 4) for controls. Their body weight ranged from 56 to 84 kg (mean 66 ± 9) for diabetics and from 47 to 80 kg (mean 67 ± 14) for controls. Blood glucose levels ranged between 10.6 and 27.2 mM (mean 17.1 ± 4.3) for patients, and the duration of disease was 2–32 yr (mean 6 ± 5). Five uncontrolled diabetic patients [4 men and 1 woman aged 29–59 yr (mean 39 ± 4) and weighing 61–80 kg (mean 70 ± 13)] were treated with insulin by means of an artificial pancreas (Biostar, Life Science, Elkhart, IN). The duration of diabetes was 2–5 yr (mean 3 ± 1), and fasting blood glucose levels were 10.6–20.4 mM (mean 15.3 ± 3.6) before and 5.8–9.6 mM (mean 7.6 ± 1.4) after installation of the artificial pancreas. The control group included 5 normal volunteers (4 men, 1 woman) aged 32–42 yr (mean 39 ± 4) and weighing 50–80 kg (mean 70 ± 13).

Studies of insulin delivery by artificial pancreas. On the day of the test, five poorly controlled type I diabetics did not receive their morning insulin injection and remained fasting until 0800 h, when blood samples were obtained. Then they were connected to an artificial pancreas that was programmed to provide insulin (Actrapid, Novo, Paris) at a variable infusion rate to maintain euglycemia for 24 h. Another blood sample was obtained the next morning at 0800 h (fasting).

Analytical techniques. Blood samples from each patient and a control subject were obtained on the same day. Blood was collected in the presence of sodium citrate (0.11 mM); leukocytes and platelets were removed as described by Beutler et al. (22). Erythrocyte membranes were prepared as described by Hanahan and Eckholm (23), and ATPase activities were measured as reported by Luly et al. (5). Assays were carried out in a final volume of 1 ml containing ~50 μg membrane protein as the enzyme source; 92 mM Tris-HCl buffer; and 5 mM $MgSO_4$, 4 mM KCl, 60 mM NaCl, 0.1 mM EDTA, and 4 mM ATP as the substrate at a final pH value of 7.5 (when stated, 0.1 mM ouabain was added to the assay mixture). Erythrocyte membranes were preincubated in the mixture for 5 min at 37°C before starting the reaction by adding the substrate. The reaction was stopped by addition of 1 ml of ice-cold trichloroacetic acid (10% wt/wt). All assays were run in triplicate. Mg^{2+}-ATPase activity is expressed as the amount of phosphorus (P_i) released in the presence of ouabain (24). Na^+-K^+-ATPase activity was calculated by subtracting the activity assayed with ouabain from that assayed without ouabain. Enzyme activity is expressed as micromoles of P_i released for 1 min per milligram protein. Proteins were estimated by the method of Lowry et al. (25) with bovine serum albumin as the standard. Reagents were obtained from Sigma (St. Louis, MO) and were of the highest available purity.

Results are expressed as means ± SD. The statistical differences were assessed by Student's *t* test.

RESULTS

ATPase activities in control subjects. Values of ATPase activities in the erythrocyte membrane of control subjects are shown in Table 1 as a function of sex. The mean values of Mg^{2+}-ATPase and Na^+-K^+-ATPase activities agree with those previously published (5). There is no difference in values of total ATPase and Mg^{2+}-ATPase activities between men and women, but Na^+-K^+-ATPase activity is increased in women ($P < .001$). Such a difference due to sex has already been described (26).

Differences in ATPase activities between type I diabetic patients and controls. Values of total, Mg^{2+}-stimulated, and Na^+-K^+-stimulated ATPase activities from patients and controls are shown in Table 2. Total ATPase activity is similar in diabetic patients and controls. Na^+-K^+-ATPase is reduced by 30% ($P < .001$), and there is a negative correlation ($r = -.76$; $P < .001$) between Na^+-K^+-ATPase activity and fasting blood glucose levels in the diabetic group (Fig. 1).

TABLE 2
ATPase activities in erythrocyte plasma membrane from control and type I diabetic subjects

Subjects	Fasting blood glucose (mM)	ATPase activities (μmol P_i · mg^{-1} protein · min^{-1})		
		Total	Na^+-K^+	Mg^{2+}
Controls	5.2 ± 0.3	1.03 ± 0.11	0.44 ± 0.06	0.58 ± 0.10
Diabetic patients	17.1 ± 4.3	1.05 ± 0.19	0.30 ± 0.10*	0.75 ± 0.17*

n = 14 in each group. For results see Table 1.
*$P < .001$.

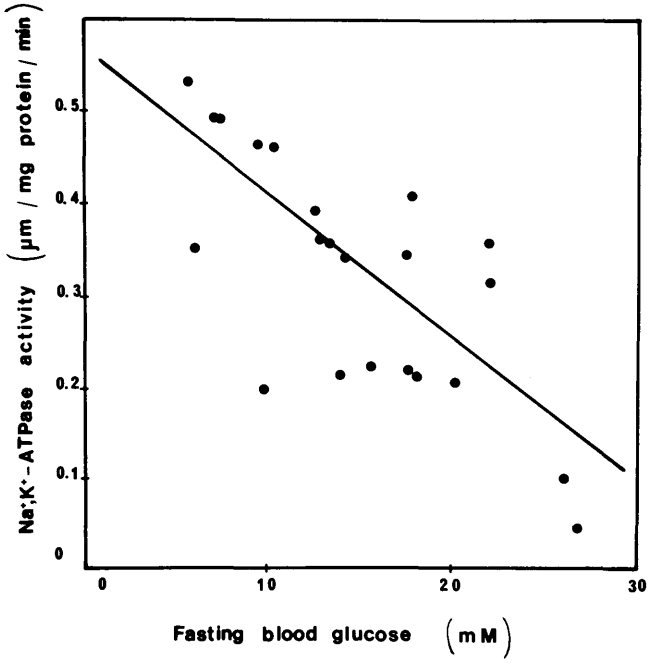

FIG. 1. Correlation between erythrocyte membrane Na^+-K^+-ATPase activity and fasting blood glucose level in type I diabetic patients ($r = -.76$, $y = 2.85 - 0.078x$, $P < .001$).

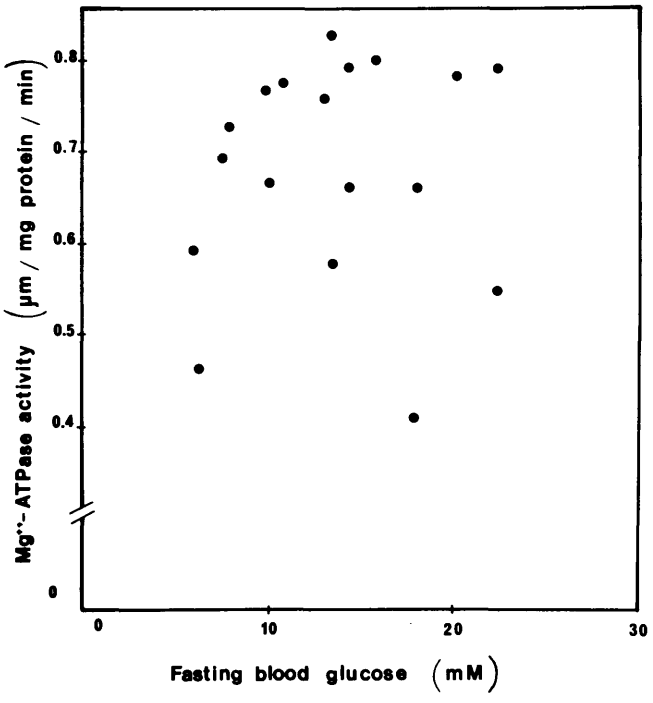

FIG. 2. Correlation between erythrocyte membrane Mg^{2+}-ATPase activity and fasting blood glucose level in type I diabetic patients ($r = .38$, $P < .05$).

The Mg^{2+}-ATPase activity is higher in patients than in controls (+29%, $P < .05$), but there is no correlation between individual values of Mg^{2+}-ATPase activity and fasting blood glucose level (Fig. 2). When only the male subjects were considered, comparable differences were found: the mean value of Na^+-K^+-ATPase activity in the diabetic patients (0.29 ± 0.11 µmol $P_i \cdot mg^{-1}$ protein $\cdot$ min^{-1}, $n = 12$) was lower ($P < .01$) than in controls (0.41 ± 0.03, $n = 8$), whereas the Mg^{2+}-ATPase activity in the diabetic patients (0.77 ± 0.17) was higher ($P < .02$) than in controls (0.59 ± 0.09).

Effect of insulin administration on ATPase activities. To study the effect of insulin on ATPase activities, assays were performed on erythrocyte ghosts from five uncontrolled patients before and 24 h after normoglycemia induced by the artificial pancreas. Before installation of the artificial pancreas and as compared with control subjects, diabetic patients showed a decrease in Na^+-K^+-ATPase activity ($P < .05$) and an increase in Mg^{2+}-ATPase activity ($P < .05$), whereas total ATPase activity remained constant. After insulin treatment, the mean fasting blood glucose level became nearly normal (7.6 ± 1.3 mM after vs. 15.3 ± 3.6 mM before treatment). ATPase activities in the treated group were not different from those in controls (Table 3). The differences observed in Na^+-K^+-ATPase and Mg^{2+}-ATPase activities before and after treatment were significant at $P < .01$ and $P < .05$, respectively. Thus, the insulin-dependent normalization of glycemia corrected erythrocyte membrane ATPase activities in these patients.

DISCUSSION

Na^+-K^+–dependent ouabain-sensitive ATPase activity, which accounts for ~40% of total ATPase activity in erythrocytes from normal subjects, only accounts for ~30% in uncontrolled diabetic patients. In contrast, Mg^{2+}-ATPase, which accounts for ~60% of total activity in controls, accounts for ~70% in diabetic patients. Comparable modifications have been described in cardiac (7), neural (18), and glomerular (19) tissues from experimental diabetic rats. Changes in phospholipid (27) and cholesterol (28) contents have been

TABLE 3
Effects of in vivo administration of insulin by artificial pancreas (AP) on ATPase activities of erythrocyte membrane in type I diabetic patients

Subjects	Fasting blood glucose (mM)	ATPase activities (µmol $P_i \cdot mg^{-1}$ protein $\cdot$ min^{-1})		
		Total	Na^+-K^+	Mg^{2+}
Controls	5.2 ± 0.3	1.06 ± 0.13	0.45 ± 0.05	0.61 ± 0.12
			$P < .05$	$P < .05$
Diabetic patients				
Before AP	15.2 ± 3.6	1.13 ± 0.21	0.32 ± 0.10	0.80 ± 0.18
			$P < .01$	$P < .05$
After AP	7.6 ± 1.3	1.11 ± 0.16	0.46 ± 0.06	0.65 ± 0.11

$n = 5$ in each group. For results see Table 1. Differences not significant except where indicated.

observed in erythrocyte membranes from diabetic patients. In addition, diabetes-dependent changes in the fatty acid composition of several tissues have been described in rats (29) and humans (16); these changes may alter the membrane lipid order (30) known to be one of the factors regulating Na^+-K^+-ATPase activity (10). On the other hand, impaired Na^+-K^+-ATPase activity has been reported to occur in hypertensive disease (31). Because diabetic patients are more susceptible to hypertension than normal subjects, it is conceivable that biochemical alterations similar to those observed in hypertension may also occur in human diabetics (21).

To know whether insulin blood level is related to the observed modifications in ATPase activity, ATPase activities were measured in patients before and after insulin treatment. Before treatment, increases in Na^+-K^+-ATPase and decreases in Mg^{2+}-ATPase activities were observed; these changes were corrected in parallel with insulinemia. These results point to a relation between insulinemia and/or glycemia and ATPase activities. The data must be analyzed with caution because of our low sample number ($n = 5$), but they are in agreement with those found in diabetic rats, where the insulin treatment normalized neural (18), glomerular (19), and myocardial (7) Na^+-K^+-ATPase activities. They are also supported by our finding of a close relationship between ATPase activity and glycemia. Insulin, within a physiologic range of concentration, has been shown to normalize lipid composition and order in erythrocytes from diabetic patients (16,17) and in ileal microvillus membranes from streptozocin-induced diabetic rats (32). One might therefore hypothesize that the membrane effect of insulin is responsible for the correction of Na^+-K^+-ATPase activity. Although the precise mechanism of this effect is uncertain, the increase in Na^+-K^+-ATPase activity by insulin may be related to the increase in monovalent cation active transport (for review see ref. 33). It has been recognized that insulin modulates the distribution of Na^+ and K^+ across the plasma membrane, an effect that was interpreted to result from either decreased permeability for Na^+ and K^+ or stimulation of Na^+-K^+-ATPase. In fact, both metabolic events strongly suggest an insulin-dependent increase in availability of pumping sites (34). The behavior of Mg^{2+}-ATPase seems to be different; even when its activity was corrected in treated patients, no correlation between glycemia and enzymatic activity was observed. This enzyme seems to be sensitive to the fatty acid composition of membranes (15) but insensitive to membrane lipid order (10). Insulin added in vitro had no direct effect on Mg^{2+}-ATPase (5), in contrast to Na^+-K^+-ATPase. Recently, Finotti and Palatini (21) reached somewhat different conclusions. As we did, they found a significant reduction in Na^+-K^+-ATPase activity, but they also observed a reduced Mg^{2+}-ATPase activity in erythrocyte membranes from uncontrolled type I diabetics. These reduced activities did not appear to be influenced by the metabolic control, as inferred from the lack of correlation between both activity values and fasting blood glucose and daily insulin dosage. Reasons for these partial differences between these results and ours are not known.

Finally, the activity of Na^+-K^+-ATPase is regulated by the phosphatidylinositol turnover in the arterial wall (35). In nerve, alterations in the regulation of Na^+-K^+-ATPase resulting from a reduction in phosphoinositide turnover appear to be a major factor in the pathogenesis of diabetic polyneuropathy (36). Therefore, it is our working hypothesis that alterations in the erythrocyte membrane of uncontrolled type I diabetic patients could account for the observed modifications in ATPase activities.

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