Calcium-Stressed Erythrocyte Membrane Structure and Function for Assessing Glipizide Effects on Transglutaminase Activation (43166A)

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Abstract. **A proposed mechanism of action of hypoglycemic sulfonylureas is the prevention of transglutaminase-mediated endocytosis of insulin receptors. When activated by high levels of intracellular calcium, transglutaminase (TG) catalyzes the cross-linking of intracellular proteins to membrane proteins and modifies membrane structure and function. This study examined the effects of the sulfonylurea glipizide on TG activity in an erythrocyte model by assessing various membrane ATPase activities and high molecular weight protein polymer formation using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. To activate TG, red blood cells were exposed to 1 m*M* intracellular Ca^{2+} using 10^{-5} *M* Ca^{2+}-ionophore A23187. In Ca^{2+}-stressed cells, calmodulin stimulation (0.1 μg/ml) of (Ca^{2+} + Mg^{2+})-ATPase was decreased to 21.2% of control activity. Increasing concentrations of calmodulin (0.1–3.0 μg/ml) could not overcome the inhibitory effects of TG on the (Ca^{2+} + Mg^{2+})-ATPase in Ca^{2+}-stressed cells with or without glipizide. An increased Ca^{2+} sensitivity of calmodulin-independent (Ca^{2+} + Mg^{2+})-ATPase due to Ca^{2+} stress was seen in all Ca^{2+}-stressed cells even in the presence of 1 m*M* glipizide. Structural changes were observed in the form of high molecular weight polymer formation. Cells exposed to high Ca^{2+} and glipizide (3×10^{-5}–10^{-3} *M*) showed no improvement in ATPase activity or protection from protein cross-linking compared with cells without the drug. We conclude that in this model glipizide fails to inhibit TG induced protein cross-linking and does not prevent the decrease in (Ca^{2+} + Mg^{2+})-ATPase activation in Ca^{2+}-stressed red blood cells. This finding considerably weakens the proposal that sulfonylureas act by inhibiting TG activity.** [P.S.E.B.M. 1991, Vol 196]

Sulfonylureas, such as glipizide, are used to lower plasma glucose levels in diabetes mellitus (1, 2). In the early stages of treatment, plasma insulin levels rise, however, as treatment progresses, insulin levels return to pretreatment levels or lower whereas the hypoglycemic effect continues to be maintained (3–6). This led to investigations of possible extra-pancreatic sites of action for the sulfonylureas. For example, several lines of research aim at understanding the mechanism behind the apparent ability of sulfonylureas to enhance insulin binding to target tissues. Others speculated that sulfonylureas may improve the transport of glucose into the cell (5), and Prince and Olefsky (7) proposed that these drugs inhibit the endocytosis of insulin receptors, thereby effectively increasing the number of binding sites for insulin. The intracellular enzyme transglutaminase (EC 2.3.2.13) (TG) has been shown to play a key role in this process of receptor-mediated endocytosis (8–10).

TG catalyzes the formation of ε-(γ-glutamyl)lysine protein cross-links when activated by 0.1–3 m*M* intracellular calcium (8, 11). This leads to extensive intracellular protein polymerization and may be responsible for the inhibition of the (Na^+ + K^+)-ATPase and calmodulin-stimulated (Ca^{2+} + Mg^{2+})-ATPase in red blood cells after prolonged exposure to higher than normal Ca^{2+} levels (12).

More recently, Gomis *et al.* (13) demonstrated that glipizide inhibits TG-mediated incorporation of

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[2,5-^{3}H]histamine into *N,N*-dimethyl casein in human red blood cell lysates both *in vivo* and *in vitro*. The *in vivo* study also showed a significant decrease in TG activity after the 45th day of treatment and a rapid return to baseline activity when glipizide treatment was discontinued.

The present study examines some of the structural and functional consequences of TG activation in a red blood cell model by assessing the $(Na^+ + K^+)$-ATPase and $(Ca^{2+} + Mg^{2+})$-ATPase activities and by measuring the high molecular weight protein polymer formation in calcium-stressed red blood cells. This approach was used to examine the effects of sulfonylureas on transglutaminase activation in a non-insulin-dependent system.

Materials and Methods

Fresh blood from healthy volunteer donors or banked blood was mixed with 154 m*M* NaCl in a ratio of 1:2.5 and centrifuged for 5 min between 3000 and 5000 rpm at 4°C. Blood lipids and white blood cells were removed by aspiration. This process was repeated three times to yield 50–60 ml of washed, packed red blood cells. A portion of the cells was then exposed to conditions appropriate for the stimulation of TG in the presence and absence of glipizide. The remaining cells served as controls.

Ten milliliters of red blood cells were mixed with 90 ml of modified Tyrode buffer containing 137 m*M* NaCl, 2.7 m*M* KCl, 1 m*M* $CaCl_2$, 5 m*M* Hepes, and 5.5 m*M* glucose (pH 6.2). The calcium ionophore A23187 (10^{-5} *M* dissolved in ethanol) was used to equilibrate intracellular with extracellular calcium levels for the activation of TG. These cells are referred to as calcium-stressed cells. Glipizide (Pfizer, Inc.), dissolved in dimethyl sulfoxide, was added to yield concentrations ranging from 30 to 1000 μmol/liter. Appropriate controls for ionophore, glipizide, and solvents were included.

All of the samples were then preincubated in a gently shaking water bath at 37°C for 4 hr and then chilled on ice. White ghost membranes were prepared according to the method of Raess *et al.* (14) and membrane protein content was determined according to the Lowry (15) protein assay. Membrane protein yields ranged from 4.0 to 8.5 mg/ml.

ATPase Assay. Membranes obtained from cells of each incubation condition were assessed for membrane $(Na^+ + K^+)$-, $(Ca^{2+} + Mg^{2+})$-, and (Mg^{2+})-ATPase activities using a semiautomated process described previously (16). Calmodulin stimulation and its concentration effect relationship (0–3.0 μg/ml) was assessed for each of the pretreated membranes, and similarly basal $(Ca^{2+} + Mg^{2+})$-ATPase activation by Ca^{2+} (0.01–0.4 m*M*) was tested in membranes from each of the incubation conditions. All ATPase activities are expressed as inorganic phosphate liberated in units of nmol/mg/min during a 60-min incubation. Under the conditions used, this is a linear reaction (not shown).

Acetylcholinesterase Assay. Ghost membranes from several experiments were evaluated for acetylcholinesterase activity to study the possible effects of Ca^{2+} loading on this particular measure of membrane integrity. Kinetic analysis of the rate of product formation was performed using the method of Steck and Kant (17). Appropriate controls for ionophore, glipizide, and ethanol were also included.

Electrophoresis. Undiluted ghost membranes were frozen and stored at −80°C. These membranes were later thawed, diluted with 20 m*M* imidazole to 4.0 or 5.0 mg protein/ml, and prepared for tube gel electrophoresis using a 4% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) (18). The electrical current during electrophoresis was held to 3 mAMP and 2–4 V/tube. Gels were fixed and stained with Coomassie brilliant blue R and then scanned at a wavelength of 562 nm and recorded using a Gilford Response spectrophotometer.

Statistical Calculations. Significance of difference in means between pretreatment groups was analyzed using Student's *t* test. Unless otherwise indicated, results are expressed as the mean ± SE. Experimental points without bars indicate errors smaller than symbol size.

Results

Membrane ATPase Activities. Figure 1 illustrates the mean of various ATPase activities of control cells, calcium-stressed cells, and calcium-stressed cells treated with 3×10^{-5} to 10^{-3} *M* glipizide. Specific activities of the (Mg^{2+})-ATPase, $(Na^+ + K^+)$-ATPase, $(Ca^{2+} + Mg^{2+})$-ATPase, and the calmodulin-activated $(Ca^{2+} + Mg^{2+})$-ATPase are shown.

There was no significant difference in the activities of the basal ATPases between the various cell conditions. However, there was a significant decrease ($P < 0.01$) in the ability of calmodulin to stimulate the $(Ca^{2+} + Mg^{2+})$-ATPase in the calcium-stressed cells compared with the control cells. As with other ATPase activities, no concentration of glipizide used showed a significant change when compared with the calcium-stressed condition in the absence of the sulfonylurea.

None of the ATPase activities was affected by the concentration of the solvents ethanol or dimethyl sulfoxide used as the vehicles for the calcium ionophore and glipizide, respectively. Furthermore, for control purposes, direct effects of sulfonylureas on ATPase activities were tested by addition of 10^{-3} to 10^{-6} *M* glyburide, tolbutamide, and chlorpropamide to the ATPase assay directly and were found to have no measurable effect on any of the ATPase activities (data not shown).

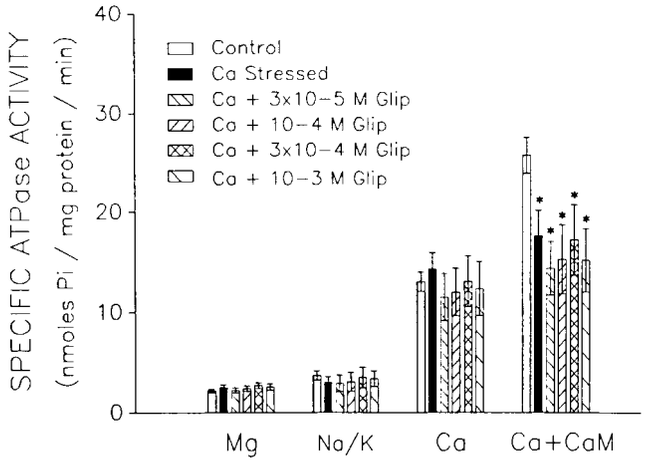

Figure 1. Membrane ATPase activities from calcium-stressed red blood cells with and without glipizide treatment. Washed human red blood cells were preincubated with 10 μM A23187, 10^{-3} M $CaCl_2$, and glipizide concentrations ranging from 3×10^{-5} to 10^{-3} M. Results are mean ± SE expressed as nmol of inorganic phosphate hydrolyzed/mg protein/min. Mg, (Mg^{2+})-ATPase; Na/K, $(Na^+ + K^+)$-ATPase; Ca, basal $(Ca^{2+} + Mg^{2+})$-ATPase; CAM, calmodulin activated $(Ca^{2+} + Mg^{2+})$-ATPase (0.1 μg of calmodulin). Control (incubation without ionophore or glipizide present): $n = 7$. Calcium stressed without glipizide: $n = 7$. Calcium stressed with glipizide: $n = 3$. *Significant difference ($P < 0.05$) compared with control cells using Student's t test.

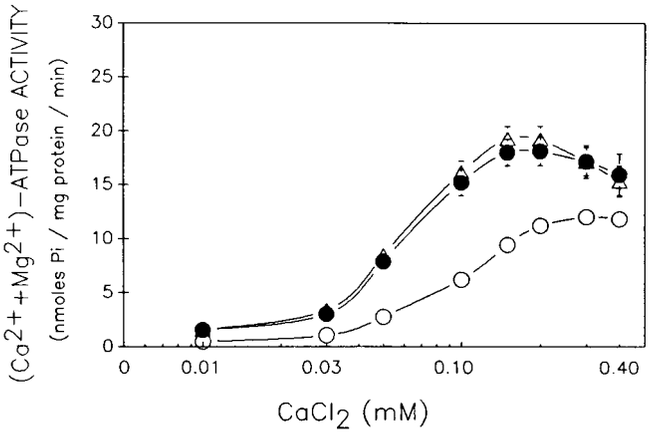

Figure 2. Calcium activation of basal $(Ca^{2+} + Mg^{2+})$-ATPase in calcium stressed red blood cells with and without 1 mM glipizide treatment. $(Ca^{2+} + Mg^{2+})$-ATPase activity was assessed with increasing doses of calcium chloride (0.01–0.4 mM) and expressed as the mean and SE of nmol of inorganic phosphate hydrolyzed/mg protein/min from six to eight determinations. Calcium-stressed cells were preincubated with 10 μM A23187 and 10^{-3} M $CaCl_2$. ○, Control, $n = 8$; ●, calcium stressed, $n = 8$; △, calcium stressed ± 1 mM glipizide, $n = 6$.

Calcium Activation of $(Ca^{2+} + Mg^{2+})$-ATPase. Figure 2 illustrates the response of the $(Ca^{2+} + Mg^{2+})$-ATPase to increasing concentrations of Ca^{2+} (0.01–0.40 mM) in the ATPase assay. Membranes used for this experiment were made from control cells (open circles), calcium-stressed cells (filled circles), and calcium-stressed cells incubated with 1 mM glipizide (triangles). At any concentration of Ca^{2+}, membranes derived from Ca^{2+}-stressed cells demonstrated a higher $(Ca^{2+} + Mg^{2+})$-ATPase activity than membranes from the control cells. Again, the presence of glipizide during Ca^{2+} exposure of the cells did not prevent the roughly 2-fold increase in the apparent Ca^{2+} affinity constant of the $(Ca^{2+} + Mg^{2+})$-ATPase.

Calmodulin Dose Response. Figure 3 demonstrates the $(Ca^{2+} + Mg^{2+})$-ATPase activity in response to increasing doses of calmodulin (0–3.0 μg/ml) added to the ATPase assay. The basal $(Ca^{2+} + Mg^{2+})$-ATPase is represented on the ordinate, where there is no exogenous calmodulin present. The data shown are from membranes prepared from control cells, calcium-stressed cells, and calcium-stressed cells incubated with 1 mM glipizide. Increasing concentrations of calmodulin did not stimulate the $(Ca^{2+} + Mg^{2+})$-ATPase in any of the calcium-stressed cells and glipizide did not overcome this apparent inhibition of calmodulin stimulation. In this experiment, the calcium-stressed cells that were not exposed to glipizide showed a higher basal $(Ca^{2+} + Mg^{2+})$-ATPase activity than either the control cells or the calcium-stressed cells incubated with glipizide.

Protein Polymer Formation. Figure 4A shows typical SDS-PAGE gels of red blood cell membrane protein profiles. Gel 1 represents membranes from the control cells, Gel 2 from calcium-stressed cells, and Gel 3 from calcium-stressed cells incubated with 1 mM glipizide, the highest concentration used in this study. The dark bands at the top of the gels on both of the calcium stressed conditions represent high molecular weight protein polymers that were too large to penetrate the gels significantly and are referred to as "polymer band." Aside from this most obvious difference in the structural profile, some more subtle changes in membrane

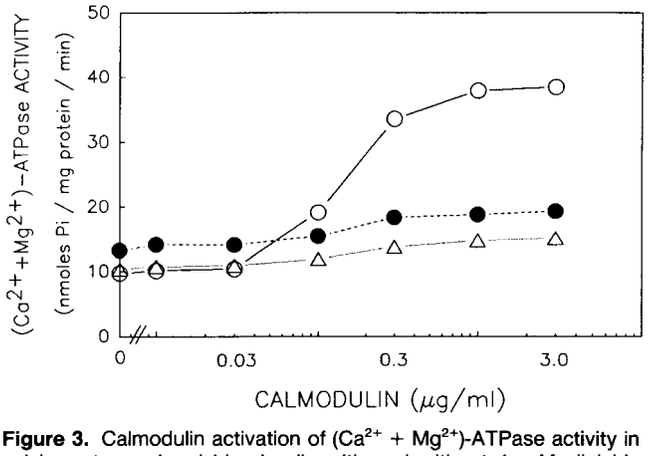

Figure 3. Calmodulin activation of $(Ca^{2+} + Mg^{2+})$-ATPase activity in calcium stressed red blood cells with and without 1 mM glipizide treatment. Calcium-stressed cells were preincubated with 10 μM A23187. $(Ca^{2+} + Mg^{2+})$-ATPase activity is expressed in nmol of inorganic phosphate hydrolyzed/mg protein/min from duplicate determinations. Data points on the ordinate represent basal $(Ca^{2+} + Mg^{2+})$-ATPase activity in the absence of added calmodulin. ○, Control, $n = 1$; ●, calcium stressed, $n = 1$; △, calcium stressed + 1 mM glipizide, $n = 1$.

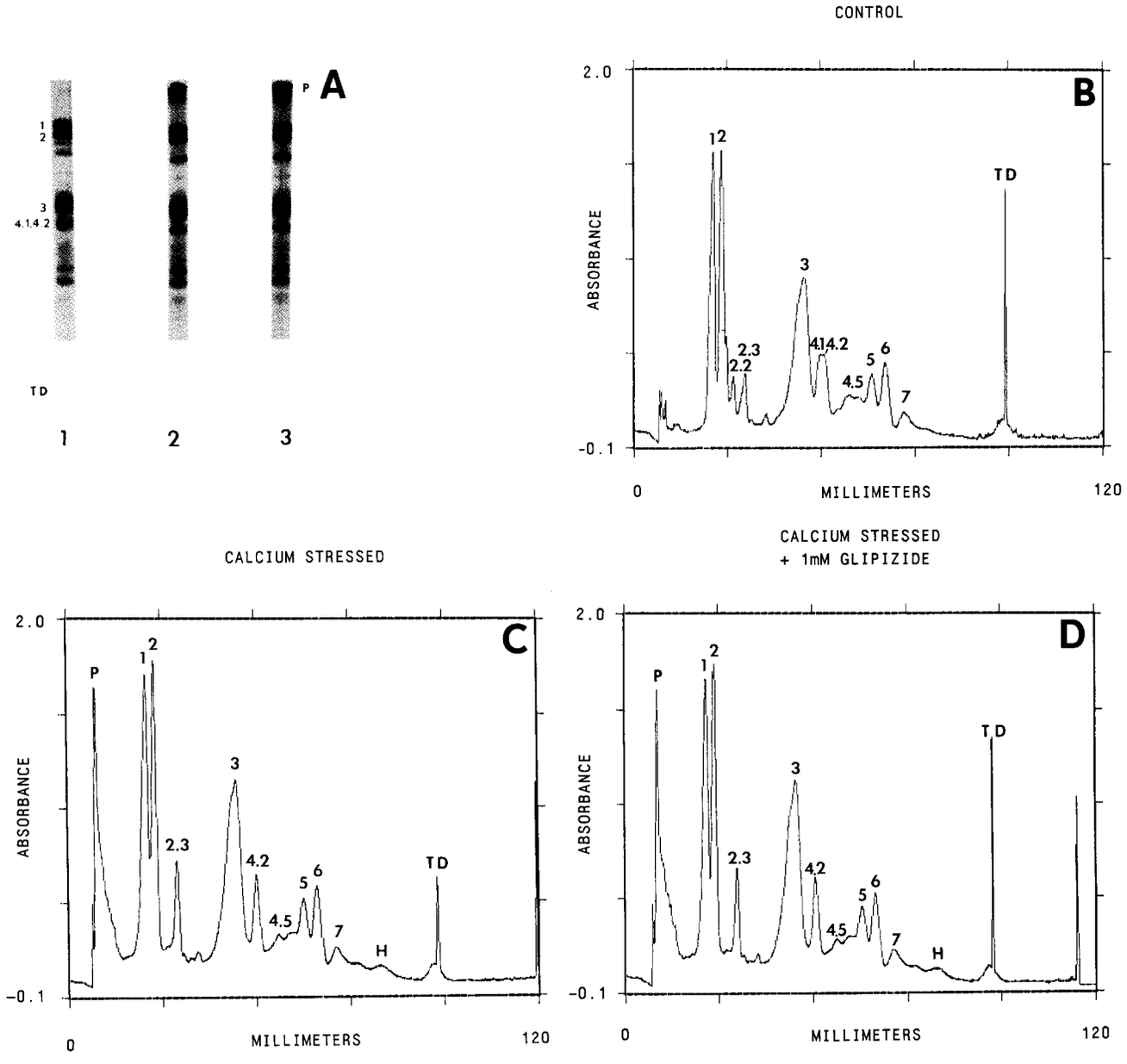

Figure 4. Membrane protein profile of calcium-stressed red blood cells with and without 1 m*M* glipizide treatment. (A) Four percent SDS-PAGE, Coomassie blue-stained gels loaded with 50 μg/tube of red blood cell membrane protein. Tube 1, membranes from control cells; Tube 2, membranes from calcium-stressed cells that were preincubated with 10 μM A23187 and 10^{-3} *M* CaCl; Tube 3, same conditions as for Tube 2 except for the addition of 1 m*M* glipizide during Ca^{2+} exposure. Polymer (P), TG induced high molecular weight protein polymers; TD, tracking dye marker. (B–D) Spectrophotometric gel scans of the membrane proteins shown in A. Gels were scanned at a wavelength of 562 nm. B, Control; C, calcium stressed; D, calcium stressed + 1 m*M* glipizide. Numerical labels correspond to the Fairbank nomenclature (18).

protein patterns can be seen. For instance, two separate protein bands appear to be diminished in both of the calcium-stressed gels when compared with the control gel. These bands correspond to ankyrin and Band 4.1.

Figure 4B simply shows the spectrophotometric scans of the gels shown in A. This type of scan was also used to quantitate the area under the curve for data shown in Table I.

Table I compares the mean (±SE) of the area under the curve of the polymer band and Band 3 obtained from spectrophotometric gel scans from six sets of duplicate gels of two independent experiments. The amount of polymer band in the membranes of calcium-stressed cells was not significantly affected by the presence of glipizide during exposure to calcium before membrane preparation ($P < 0.05$).

Acetylcholinesterase Activity. In two sets of membrane preparations, control cells had a 2.5–2.8 greater acetylcholinesterase activity than did calcium-stressed cells. This change was not affected by the presence of 2×10^{-4} or 10^{-3} *M* glipizide (data not shown).

Table 1. SDS-PAGE Profile of Selected Membrane Proteins from Calcium-Stressed[a] Red Blood Cells with and without Glipizide Pretreatment

	Control	Ca^{2+}-Stressed glipizide (*M*)				
		0	3×10^{-5}	10^{-4}	3×10^{-4}	10^{-3}
Polymer band	1.23 ± 0.24	4.88 ± 0.10[b]	5.78 ± 0.35[b,c]	6.26 ± 1.06[b,c]	5.41 ± 0.43[b,c]	5.63 ± 0.06[b]
Band 3	7.26 ± 0.84	8.86 ± 0.11	9.17 ± 0.47	9.91 ± 1.77	9.27 ± 0.29	9.74 ± 0.70
Polymer:Band 3	0.17	0.55	0.63	0.63	0.58	0.58

Note: Values represent mean and SE of the area under the curve from six sets of duplicate gels of two independent experiments.
[a] Calcium-stressed cells were preincubated with 10 μM A23187.
[b] Significantly different from control cells by Student's *t* test ($P < 0.05$).
[c] Not significantly different from value of calcium stressed cells not pretreated with glipizide ($P > 0.05$).

Discussion

Several studies have sought to define the extrapancreatic effects of sulfonylureas used in the treatment of Type II diabetes. Some of the studies that have examined the effects of sulfonylureas on insulin receptors have produced conflicting results. For instance, Prince and Olefsky (7) found that glyburide, a second generation sulfonylurea, increased the number of insulin receptors on the surface of human fibroblasts. In the presence of insulin, glyburide inhibited the insulin-induced loss of receptors by 34% (7). However, an *in vivo* study with glipizide showed no change in insulin levels, number of insulin receptors, or TG activity in rat hepatocytes when compared with control cells, although there was a hypoglycemic effect (5). These authors concluded that the action of sulfonylureas may occur after the binding of insulin to its receptor, perhaps in the transport of glucose into the cell. To our knowledge, it has not yet been resolved whether the discrepancies between these studies lie in the use of different cell types. Important to the design of both of these studies, however, is the hypothesis that sulfonylureas may exert their hypoglycemic action by inhibiting the enzyme TG. This enzyme has been implicated as the key enzyme involved in receptor-mediated endocytosis (8–10). Such an inhibition of TG would effectively increase the number of receptors available to bind with insulin to maintain glucose homeostasis, and recently Gomis *et al.* (13) have demonstrated an inhibition of TG activity in human red blood cell lysates by glipizide, both *in vitro* and *in vivo*.

The present study was designed to determine whether glipizide has an inhibitory effect on TG-induced cellular changes using the human red blood cell as a model system. This is a non-insulin-sensitive system in which TG activation can be induced by 0.1–3 m*M* intracellular calcium (8, 11) and the functional consequences of TG activity can be assessed by measuring membrane-associated ATPase activities and the formation of high molecular weight protein polymers.

Results from both the ATPase and electrophoresis experiments indicate that TG was indeed activated in the calcium-stressed cells. There is a 79% decrease of the calmodulin-stimulated (Ca^{2+} + Mg^{2+})-ATPase and increased formation of protein polymers in the cells exposed to higher than normal levels of intracellular calcium compared with the control cells. These changes were statistically significant at $P < 0.01$ and $P < 0.05$, respectively.

The decrease in the ability of calmodulin to stimulate the basal (Ca^{2+} + Mg^{2+})-ATPase of the calcium-stressed cells, shown in Figure 1, suggests that the ATPase was already close to maximum stimulation before the application of exogenous calmodulin. This observation is further supported by the data in the calcium dose-response curve (Fig. 2), which shows that all of the calcium-stressed cells had a greater basal (Ca^{2+} + Mg^{2+})-ATPase activity with every concentration of Ca^{2+} added to the ATPase assay. In fact, even increasing doses of exogenous calmodulin applied to the ATPase assay (Fig. 3) could not stimulate the (Ca^{2+} + Mg^{2+})-ATPase in the calcium-stressed cells.

Several possible explanations for these observations exist. One is that the endogenous calmodulin was covalently cross-linked by TG to the ATPase, which would preclude it from being removed during the hemolysis step using chelator EGTA. Alternatively, a membrane-associated Ca^{2+}-dependent protease may have detached an inhibitory domain from the ATPase and it may also have cleaved the calmodulin binding site from the (Ca^{2+} + Mg^{2+})-ATPase (19). This is a less likely possibility since we used freshly drawn human cells, which are loaded with endogenous protease inhibitors such as calpastatin.

As can be seen in Figure 1, no concentration of glipizide prevented the inhibition of calmodulin stimulation in calcium-stressed cells. Furthermore, the presence of 1 m*M* glipizide in the incubation of the calcium-stressed cells did not affect the response of the (Ca^{2+} + Mg^{2+})-ATPase to increasing concentrations of calcium or calmodulin applied to the ATPase assay (Figs. 2 and 3).

Acetylcholinesterase activity was also decreased in the calcium-stressed cells compared with the control cells. This decrease was almost 3-fold (data not shown).

The acetylcholinesterase activity was evaluated as a possible alternative means of normalizing cells from the various incubation conditions. However, the enzyme activity was consistently decreased in the calcium-stressed cells on a mg membrane protein/ml basis. This finding may be a result of diminished activity of the enzyme or loss of the enzyme due to conformational changes of the membrane during the incubation with high levels of intracellular calcium. Alternatively, the enzyme activity may be artifactually low (on a per mg protein basis) due to cross-linking of intracellular proteins such as hemoglobin. Regardless of the mechanisn involved, the presence of glipizide in the incubation of the calcium-stressed cells did not affect the acetylcholinesterase activity.

Data from the electrophoresis of the membranes of control cells and calcium-stressed cells provide additional evidence that TG was activated under the experimental conditions. Figure 4A clearly shows considerable protein polymer formation in both of the gels made from membranes exposed to high intracellular calcium, suggesting that cross-linking of membrane proteins did occur.

It is not clear from this study precisely which proteins were cross-linked to form the polymers; however, two protein bands appear to be diminished in the calcium-stressed gels compared with the control gels. These bands are ankyrin and Band 4.1. This finding is intriguing because the two proteins share common functional characteristics. Both interact with spectrin, a peripheral membrane protein, and each interacts with an integral membrane protein: ankyrin interacts with Band 3, whereas 4.1 interacts with glycophorin. Therefore, both ankyrin and Band 4.1 anchor spectrin to the membrane via an interaction with an integral membrane protein (20). These considerations are in good agreement with recent immunoblotting and immunoelectrophoretic studies using calcium-activated TG in human erythrocytes, which show evidence of spectrin, ankyrin, Band 3, and Band 4.1 in the polymer along with glucose transporter, actin, and hemoglobin (21).

This study used various approaches to investigate the role of glipizide on some of the functional consequences of calcium-activated TG in the red blood cell. Thus, all parameters tested in this study yielded results that indicate that glipizide has no effect on TG-linked activities. Our findings are in contrast to those of Gomis *et al.* (13), who found that 10^{-3} *M* glipizide inhibited TG activity in human red blood cell lysates by as much as 20%. A possible explanation of these results and the present study is that a 20% inhibition of TG by glipizide may not be enough of an inhibition to manifest a change in the functional consequences of the red blood cell enzyme. However, if this reasoning is carried one step further, then it could be speculated that a 20% inhibition of TG might have no effect on the internalization of insulin receptors—another functional consequence of TG activity. On the other hand, it could be that a 20% inhibition of the enzyne may bring about a significant consequence in terms of receptor internalization, but not in the parameters used in this study. In other words, the process of receptor-mediated endocytosis may be more sensitive to inhibition of TG than are polymer formation or inhibition of calmodulin-stimulated (Ca^{2+} + Mg^{2+})-ATPase activity examined in this study.

It is equally possible that polymer formation may not be a sensitive enough measure of limited TG activity. Likewise, the decrease in calmodulin stimulation of the (Ca^{2+} + Mg^{2+})-ATPase may not be mediated by TG. For instance, calcium-dependent protease activity could be responsible for the altered membrane protein profiles and the inhibition of calmodulin stimulation of the basal (Ca^{2+} + Mg^{2+})-ATPase in the calcium-stressed cells (19). Au *et al.* (19) found evidence suggesting that a Ca^{2+}-dependent membrane-associated protease stimulates the (Ca^{2+} + Mg^{2+})-ATPase after only 30-min incubation with Ca^{2+}. However, they saw no evidence of membrane degradation until after an overnight incubation at 37°C. In the present study, polymer formation and a diminished quantity of ankyrin and Band 4.1 were observed after a 4-hr incubation with Ca^{2+}. It is unlikely that a protease could have been responsible for these changes, because Band 3 was not degraded and there was no evidence of low molecular weight protein accumulation at the bottom of the tube gels, although a calpastatin-insensitive protease activity cannot be discounted entirely. This is interesting in light of observations by others (22, 23), using a somewhat different membrane preparation, who demonstrate cross-linking as well as proteolysis of Band 3 in Ca^{2+}-stressed red blood cells.

The results from this study indicate that glipizide does not inhibit two processes thought to be mediated by TG in red blood cells. This finding considerably weakens the hypothesis that glipizide and other sulfonylureas exert their extra-pancreatic effects by inhibiting TG activity, and indicates that alternative explanations for drug-induced changes in receptor populations need to be explored.

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1. Zimmerman BR, Service FJ. Management of noninsulin-dependent diabetes mellitus. Med Clin North Am 72:1355–1364, 1988.
2. Ferner RE. Oral hypoglycemic agents. Med Clin North Am 72:1323–1335, 1988.
3. Feldman JM, Lebovitz HE. Endocrine and metabolic effects of

glybenclamide: Evidence for an extrapancreatic mechanism of action. Diabetes **20**:745–655, 1971.
4. Pfeifer MA, Halter JB, Judzewitsch RG, Beard JC, Best JD, Ward WK, Porte D Jr. Acute and chronic effects of sulfonylurea drugs on pancreatic islet function in man. Diabetes Care **7**(suppl 1):25–34, 1984.
5. Nowak SM, McCaleb ML, Lockwood DH. Extrapancreatic action of sulfonylureas: Hypoglycemic effects are not dependent of altered insulin binding or inhibition of transglutaminase. Metabolism **32**:398–402, 1983.
6. Kolterman OG, Olefsky JM. The impact of sulfonylurea treatment upon the mechanisms responsible for the insulin resistance in type II diabetes. Diabetes Care **7**(suppl 1):81–88, 1984.
7. Prince MJ, Olefsky JM. Direct *in vitro* effect of a sulfonylurea to increase human fibroblast insulin receptors. J Clin Invest **66**:608–611, 1980.
8. Folk JE. Transglutaminases. Ann Rev Biochem **49**:517–531, 1980.
9. Levitzki A, Willingham M, Pastan I. Evidence for participation of transglutaminase in receptor-mediated endocytosis. Proc Natl Acad Sci USA **77**:2706–2710, 1980.
10. Davies PJA, Davies DR, Levitzki A, Maxfield FR, Milhaud P, Willingham MC, Pastan IH. Transglutaminase is essential in receptor-mediated endocytosis of alpha-2-macroglobulin and polypeptide hormones. Nature **283**:162–167, 1980.
11. Siefring GE Jr, Apostol AB, Velasco PT, Lorand L. Enzymatic basis for the Ca^{2+}-induced cross-linking of membrane proteins in intact human erythrocytes. Biochemistry **17**:2598–2604, 1978.
12. Vincenzi FF, Raess BU. Red blood cell calcium pump ATPase activity: Reversible and irreversible changes caused by intracellular calcium. Fed Proc 30:304, 1979.
13. Gomis R, Casanovas A, Casamijtana R, Sarto A, Arroyo J, Coves MJ, Rivera F, Vilardell E. The effect of hypoglycemic sulfonylureas on human red blood cell transglutaminase activity. Diab Res Clin Pract 5:31–36, 1988.
14. Raess BU, Record DM, Tunnicliff G. Interaction of phenylglyoxal with the human erythrocyte (Ca^{2+} + Mg^{2+})-ATPase: Evidence for the presence of an essential arginyl residue. Mol Pharmacol **27**:444–450, 1985.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
16. Raess BU, Vincenzi FF. A semi-automated method for the determination of multiple membrane ATPase activities. J Pharmacol Methods **4**:273–283, 1980.
17. Steck TL, Kant JA. Preparation of impermeable ghosts and inside-out vesicles from human erythrocyte membranes. Methods Enzymol **31**:172–180, 1974.
18. Fairbanks G, Steck TL, Wallach DFH. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10**:2606–2617, 1971.
19. Au KS, Lee MF, Siu YL. Ca^{2+}-mediated activation of human erythrocyte membrane Ca-ATPase. Biochim Biophys Acta **978**:197–202, 1989.
20. Guidotti G. Membrane proteins: Structure, arrangement, and disposition in the membrane. In: Andreoli TE, Hoffman JF, Fanestil DD, Schultz SG, Eds. Physiology of Membrane Disorders. New York: Plenum Publishing Corp., 1986.
21. Bjerrum OJ, Heegaard NH. Has immunoblotting replaced electroimmunoprecipitation? Examples from the analysis of autoantigens and transglutaminase-induced polymers of the human erythrocyte membrane. J Chromatogr **470**:351–367, 1989.
22. Lorand L, Bjerrum OJ, Hawkins M, Lowe-Krentz L, Siefring GE Jr. Degradation of transmembrane proteins in Ca^{2+}-enriched human erythrocytes. An immunochemical study. J Biol Chem **258**:5300–5305, 1983.
23. Lorand L, Michalska M. Altered response of stored cells to Ca^{2+} stress. Blood **4**:1025–1027, 1985.