Low pH and Ketoacids Induce Insulin Receptor Binding and Postbinding Alterations in Cultured 3T3 Adipocytes

J. P. M. VAN PUTTEN, Tj. WIERINGA, AND H. M. J. KRANS

SUMMARY

The etiology of insulin resistance during diabetic ketoacidosis is still poorly understood. Changes in insulin receptor binding and the existence of postreceptor alterations have been proposed. In an attempt to clarify the role of low pH and ketone bodies in the insulin resistance, we examined the effectiveness of insulin during and after 48 h of exposure of cultured 3T3-L_1 adipocytes to low pH and ketoacids. In the "acute" stage, lowering of physiologic pH (pH 7.4) to pH 6.9 induced a decrease in insulin binding (50%), which was due to a decrease in the rate of association. Concomitantly, the insulin sensitivity was decreased (ninefold). The basal hexose uptake and insulin responsiveness were only slightly decreased at low pH. Beta-hydroxybutyrate partially counteracted the effect of low pH on insulin binding and sensitivity in a dose-dependent fashion (ED_{50}: 10 mM). The binding-enhancing effect of ketoacids was more pronounced at low pH than at physiologic pH and absent at optimum pH (pH 8.0). After 48 h of exposure of the cells to pH 6.9, insulin binding and insulin sensitivity (measured at physiologic pH) were similar as in cells cultured at pH 7.4. The insulin response, however, was substantially impaired (40%), due to an increase in basal hexose uptake as well as a decrease in maximal insulin-stimulated uptake. These postbinding alterations induced by low pH could be reversed by culturing the cells at physiologic pH for another 48 h. Prolonged exposure to ketoacids did not affect the insulin effectiveness. Our data suggest that the insulin resistance accompanying ketoacidosis is due to low pH-induced insulin receptor binding as well as postbinding alterations. Ketoacids do not seem to contribute to the insulin resistance. **DIABETES 1985; 34:744–50.**

From the Laboratory for Diabetes Research, Department of Endocrinology and Metabolic Diseases, University Hospital, 2333 AA Leiden, The Netherlands.
Address reprint requests to Prof. Dr. H. M. J. Krans, Department of Endocrinology and Metabolic Diseases, University Hospital, Bldg. 30, 2333 AA Leiden, The Netherlands.
Received for publication 10 February 1984 and in revised form 30 December 1984.

The existence of insulin resistance during diabetic ketoacidosis has been well established.[1–6] Its etiology, however, is still poorly understood. A role of counterregulatory substances[6] as well as an effect of low pH and ketoacids by themselves have been suggested. Roth et al.[2] speculated that the pH dependence of the binding step contributes to insulin resistance. Low pH reduces insulin binding.[7–12] The effect of ketoacids on the insulin binding is uncertain: both an increase[8,11,13] and no alteration[14] in binding have been reported. Whether alterations in insulin binding fully account for the insulin resistance accompanying diabetic ketoacidosis is unclear. In most studies, only binding and no biologic effect of insulin have been determined. Hidaka et al.[11] did study the effects of pH and ketoacids on insulin binding and insulin action in cultured human fibroblasts. Surprisingly, induced changes in insulin binding were only partially reflected in the effect of insulin. In vivo, ketoacidosis may last for several days. Yet, the influence of the duration of the ketoacidosis on the insulin effectiveness is unknown. Studies in rats suggest the existence of postreceptor alterations during acidemia.[3]

The lack of knowledge about the etiology of insulin resistance during ketoacidosis prompted us to systematically study the acute and long-term effects of low pH (pH 6.9) and ketoacids on the insulin receptor binding and the insulin-sensitive hexose uptake in cultured 3T3 adipocytes. Our results demonstrate that acute exposure to low pH and ketoacids primarily induces alterations in insulin receptor binding, whereas after prolonged incubation postbinding alterations are predominant.

MATERIALS AND METHODS

Cell culture. 3T3-L_1 preadipocytes (Flow Lab, Irvine, United Kingdom), a cloned line of 3T3 cells established by Green et al.,[15] were plated at a density of 3×10^3 cells/cm^2 and grown to confluence in Dulbecco's modified Eagle's medium (25 mM glucose) containing fetal bovine serum (10% vol/

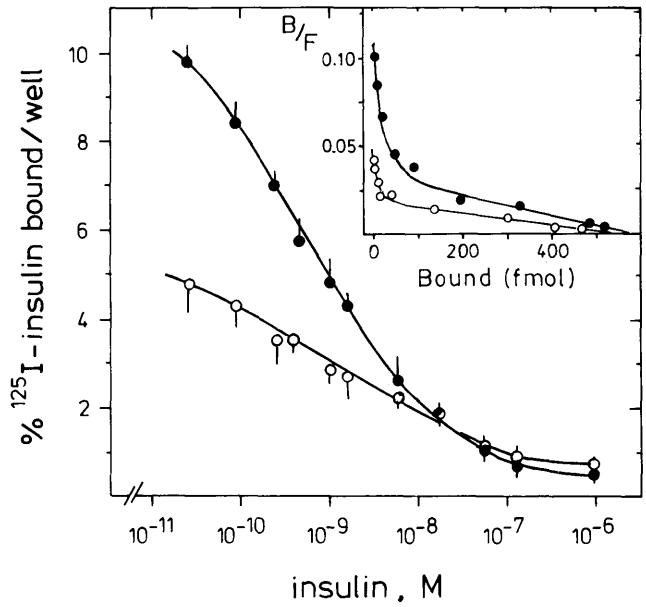

FIGURE 1. Displacement curve of ^{125}I-insulin binding. Fat cells were incubated at pH 6.90 (○) and pH 7.40 (●) with 20 pM ^{125}I-insulin and increasing amounts of unlabeled insulin for 3 h at 20°C. Inset: Scatchard plot of the binding data.

vol), L-glutamine (2 mM), and antibiotics (standard medium). Cells were kept at 37°C in a humidified atmosphere of 7.5% CO_2 in air, and were fed every other day. Two days postconfluence, the adipose conversion was enhanced according to the method of Rubin[16] as modified by Reed and Lane.[17] Standard medium was supplemented with insulin (10 μg/ml), dexamethasone (0.25 μM), and 1-methyl-3-isobutylxanthine (0.5 mM) for 2 days and with insulin alone for 6 days. After this procedure, 80–90% of the cells expressed the adipocyte phenotype. Before starting experiments, fat cells were maintained in culture medium without insulin for at least 3 days.

Insulin binding. ^{125}I-insulin binding experiments were performed with 3T3 adipocytes adherent to the dishes (60 mm). Dishes were rinsed three times with 2 ml Dulbecco's phosphate-buffered saline (PBS), pH 7.40, and subsequently incubated in 1.5 ml Krebs-Ringer Tris-HCl buffer (118 mM NaCl, 1.3 mM $MgSO_4$, 4.8 mM KCl, 1.85 mM $CaCl_2$, and 50 mM Tris) containing 2% BSA and adjusted to pH 6.90 or pH 7.40 (20°C). Bacitracin (1.5 mM) was present to inhibit insulin degradation. Then, 20 pM mono-^{125}I-insulin (3200 cpm/fmol) was added and the plates were shaken (75 oscillations/min) at 20°C. After 3 h, when binding had reached steady state under all conditions employed, the dishes were rapidly rinsed four times with 2 ml ice-cold PBS. Cells were scraped off the dishes, solubilized in 1 ml of 0.1 M NaOH, and divided into aliquots for protein determination[18] and ^{125}I counting. Nonspecific binding, defined as the amount of insulin bound in the presence of 10^{-6} M native insulin, was subtracted (unless otherwise indicated). The association of ^{125}I-insulin was studied by stopping the binding experiment at selected time intervals. To study the dissociation of ^{125}I-insulin, a large volume (0.2 ml/cm^2) of insulin-free Tris buffer was added to the cells after attainment of binding equilibrium. Every 10–15 min buffer was replaced by fresh buffer, and the amount of ^{125}I dissociated into the fractions was counted. To distinguish association and dissociation of insulin from internalization and efflux of internalized label, some experiments were also performed at 15°C, at which temperature internalization and efflux of label has been shown to be minimal.[19–22] Similar results were obtained at 20°C and 15°C, indicating that true receptor association and dissociation were measured.

Hexose uptake was measured using the glucose analogue 2-deoxy-D-glucose (dGlc). dGlc is transported and phosphorylated like glucose, but not further metabolized. Cells (30-mm wells) were rinsed three times with 1 ml PBS (37°C) and incubated in 0.75 ml Tris buffer at pH 6.90 or pH 7.40, at 37°C. Cells were preincubated with or without insulin for 30 min. 2-Deoxy-D-(1-^{14}C)-glucose uptake (0.1 mM, 710 cpm/nmol, unless otherwise indicated) was measured during intervals (see figure legends) in which uptake was linear with time. During this period, the intracellular nonphosphorylated dGlc concentrations were always below the extracellular concentration, indicating that transport was rate limiting in the uptake process. Transport was stopped by four 1-ml washes with ice-cold PBS. Cells were dispatched as for insulin binding, and radioactivity was counted in a liquid scintillation

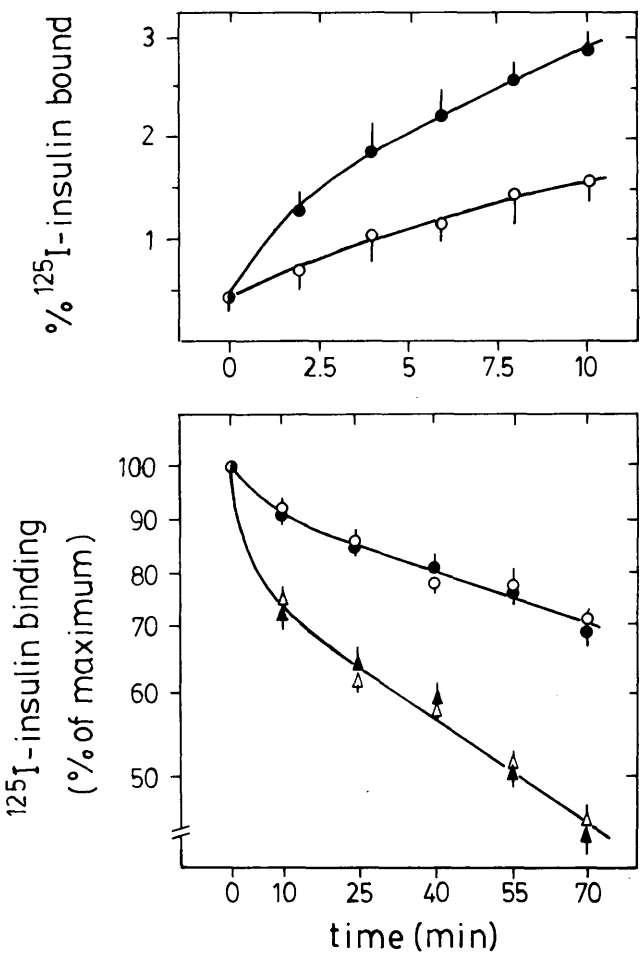

FIGURE 2. Effect of pH on insulin binding kinetics. The association (upper panel) of ^{125}I-insulin (20 pM) was measured at pH 6.90 (○) and pH 7.4 (●) (20°C). The dissociation of ^{125}I-insulin (lower panel) was determined in the absence (○,●) and presence (△,▲) of 16.6 nM native insulin. Cells were allowed to bind ^{125}I-insulin (20 pM) for 3 h at pH 7.40 (20°C). Subsequently, the dissociation was studied at pH 6.90 (○,△) and pH 7.40 (●,▲).

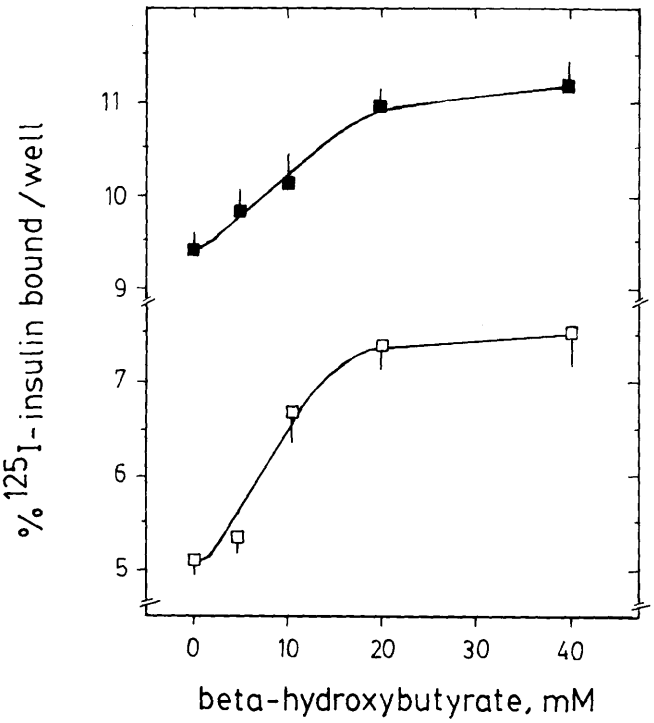

FIGURE 3. Enhancement of insulin binding by beta-hydroxybutyrate. Cells were allowed to bind ^{125}I-insulin (20 pM) at pH 6.90 (□) and pH 7.40 (■) for 3 h at 20°C in the presence of various concentrations of DL-beta-hydroxybutyrate.

counter. Non-cytochalasin B (100 μM)-inhibitable dGlc uptake was < 2% of the total uptake and only taken into account in the calculation of the transport kinetics.

Data analysis. Data are expressed as the mean ± SEM of separate experiments. Where appropriate, the data were statistically analyzed by Student's paired *t*-test.

Materials. Media, sera, and L-glutamine were obtained from Gibco (Grand Island, New York). Penicillin and streptomycin were purchased from Gist-Brocades (Delft, The Netherlands). Bovine serum albumin (BSA) was from Organon (Oss, The Netherlands). Mono-^{125}I-insulin (SA 360 mCi/mmol, porcine and human) was a generous gift of Eli Lilly Nederland (Utrecht, The Netherlands). Native insulin was from Novo (Copenhagen, Denmark). 2-Deoxy-(1-^{14}C)-D-glucose was obtained from New England Nuclear (Boston, Massachusetts). Bacitracin, 2-deoxy-glucose, and cytochalasin B were from Sigma (St. Louis, Missouri). All other chemicals were of p.a. grade.

RESULTS

Acute effects of low pH and ketoacids on insulin effectiveness. The acute effect of low pH on insulin binding was determined by comparing ^{125}I-insulin binding at pH 6.90 and pH 7.40 (physiologic pH). At low pH, ^{125}I-insulin binding was decreased by up to 50% ($P < 0.001$, Figure 1). It should be pointed out that in most studies of ^{125}I-insulin binding, experiments are performed at pH 7.6–8.0 to optimize conditions. In comparison to binding at optimum pH (12.3 ± 0.2% ^{125}I-insulin bound/well at pH 8.0), the binding at physiologic pH was low and at pH 6.90 extremely low. Scatchard analysis of the binding data[19] revealed that the decrease in binding with lowering of pH was due to a change in receptor affinity rather than to alterations in receptor number (159,000 and 175,000 binding sites per cell at pH 6.90 and pH 7.40, respectively; not statistically significant; Figure 1, inset). A decrease in receptor affinity might result from a reduction in the association rate and/or an increase in the rate of dissociation of the hormone from the receptor. In the cultured adipocytes, low pH decreased the rate of association of ^{125}I-insulin to its binding sites ($P < 0.005$, Figure 2, upper panel). The rate of dissociation was not affected by low pH. Furthermore, in the presence of unlabeled insulin (100-fold concentration), the rate dissociation was increased both at pH 6.90 and pH 7.40 to a similar extent (Figure 2, lower panel). These data indicate that the decrease in binding at low pH is primarily a consequence of a reduction in the rate of association.

The acute effects of ketoacids on ^{125}I-insulin binding were determined by adding a buffered solution of DL-beta-hydroxybutyrate (5–40 mM) to the incubation buffer. Beta-hydroxybutyrate increased the ^{125}I-insulin binding in a dose-dependent fashion (ED_{50}: 10 ± 1.3 mM; Figure 3; $P < 0.01$ at 10 mM). The increase in binding was more pronounced at pH 6.90 than at pH 7.40 (47% versus 27%) and therewith partially counteracted the effect of low pH on ^{125}I-insulin binding. At optimum pH for binding (pH 8.0), beta-hydroxybutyrate hardly influenced insulin binding (not shown). Equivalent concentrations (0–20 mM) of sucrose or NaCl did not mimic the effect of beta-hydroxybutyrate (not shown). The binding-enhancing effect of the compound, therefore, does not seem to be due to nonspecific osmotic or ionic effects.

The acute effects of pH and ketoacids on insulin action were determined by measuring the insulin-sensitive 2-deoxyglucose (dGlc) uptake by cultured fat cells at pH 6.90 and pH 7.40. At low pH, uptake of dGlc both in the absence (basal uptake) and presence of insulin was slightly decreased (Figure 4, $P < 0.05$). The insulin sensitivity was markedly decreased (ED_{50}: 0.4 ± 0.1 nM versus 3.8 ± 0.3 nM at pH 7.40

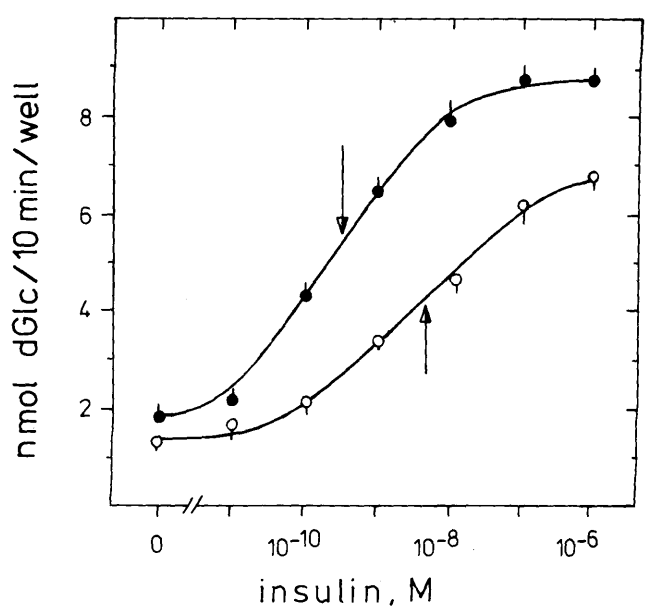

FIGURE 4. Dose-response relationship of the insulin-sensitive dGlc uptake (10 min, 37°C) at pH 6.90 (○) and pH 7.40 (●). The arrows indicate the half-maximal effective concentration of insulin.

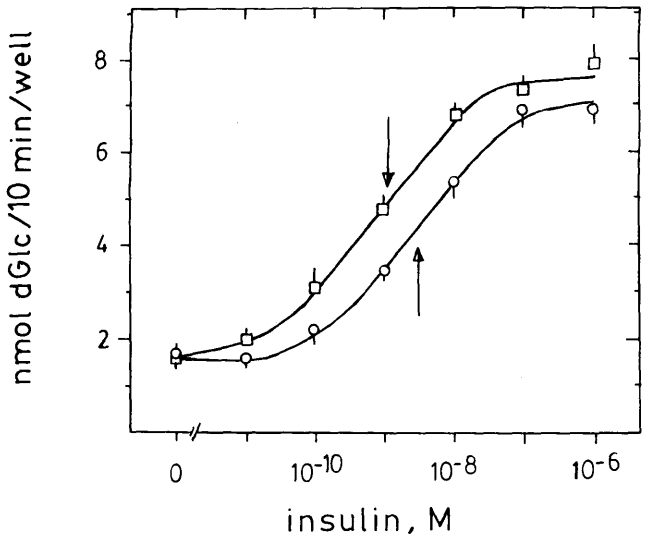

FIGURE 5. Effect of beta-hydroxybutyrate on the insulin-sensitive dGlc uptake. dGlc uptake (10 min, 37°C) was measured in the absence (○) and presence (□) of 20 mM beta-hydroxybutyrate at pH 7.40. The arrows indicate the half-maximal effective concentrations of insulin.

versus pH 6.90, respectively; $P < 0.001$). Beta-hydroxybutyrate (5–40 mM) did not affect the basal and maximal insulin-stimulated dGlc uptake (Figure 5), although in some experiments an increase in insulin responsiveness was observed (not statistically significant). At submaximal concentrations of insulin, however, the insulin response was increased (at pH 6.90, ED_{50}: 1.2 ± 0.2 nM versus 3.8 ± 0.3 nM; $P < 0.05$), which might (at least partially) be a reflection of the increase in insulin binding.

Long-term effects of low pH and ketoacids on insulin effectiveness. To assess the long-term effect of low pH on insulin binding, fat cells were cultured in standard medium at pH 6.90 and pH 7.40 for 48 h. Then, ^{125}I-insulin binding was determined at physiologic pH (pH 7.40). Restoration of pH 7.40 after 48 h of incubation at low pH normalized the insulin-receptor interaction instantly (not shown). The presence of various concentrations of DL-beta-hydroxybutyrate during the incubation period (48 h) did not affect the subsequently measured ^{125}I-insulin binding either. So both low pH and ketoacids did not induce long-term alterations in ^{125}I-insulin binding.

The effect of prolonged incubation at low pH on insulin action was determined by measuring the insulin-sensitive dGlc uptake at pH 7.40 subsequent to 48 h of maintenance of the cells in medium at pH 6.90. As shown in Figure 6, prolonged incubation at low pH increased the basal dGlc uptake (35 ± 4%, $P < 0.01$). The maximal insulin-stimulated dGlc uptake on the other hand was decreased (23 ± 5, $P < 0.01$). Consequently, the insulin response, expressed as percentage stimulation of basal dGlc uptake, was reduced drastically (230% versus 480%). The insulin sensitivity was not changed by prolonged incubation at low pH, which is compatible with the absence of long-term alterations in insulin binding. Prolonged incubation of the fat cells with DL-beta-hydroxybutyrate (5–40 mM) did not influence the subsequently measured insulin response (Figure 7).

The postbinding alterations induced by prolonged exposure of the cultured fat cells to low pH were studied in more detail, since they might contribute to the insulin resistance accompanying ketoacidosis. The time course of the effect of low pH on the basal and maximal insulin-stimulated dGlc uptake is shown in Figure 8. The increase in basal dGlc uptake and the decrease in insulin responsiveness were maximal within 48 h. Incubation for 72 h did not result in a further reduction of the insulin effectiveness, whereas during longer periods of incubation the cells deteriorated and detached from the dishes. The reversibility of the long-term alterations was studied by culturing the cells first at pH 6.90 for 48 h, and, subsequently, at pH 7.40. The long-term effects of low pH were slowly reversible (Figure 8). In about 48 h, the basal dGlc uptake and the insulin responsiveness were normalized.

The low pH-induced increase in basal dGlc uptake might be due to an increase in the number or activity of the hexose transporters as well as to an increase in the affinity of the hexose transporters for the hexose. To discriminate between these possibilities, a concentration curve of the dGlc uptake was performed. Lineweaver-Burke analysis of the data (Table 1) revealed that the increase in basal hexose uptake was due to an increase in the apparent V_{max} rather than to a change in the apparent K_m of the hexose transport system. This indicates that after a long-standing period of low pH, hexose uptake is increased by an increase in the number or activity of hexose transporters rather than by a change in affinity.

DISCUSSION

In an attempt to understand more about the etiology of insulin resistance accompanying ketoacidosis, the acute and long-term effects of low pH and ketoacids on the insulin binding and the insulin-sensitive dGlc uptake were studied in cultured 3T3 fat cells. In the acute stage, low pH primarily induced alterations in insulin receptor binding. At pH 6.90, ^{125}I-insulin

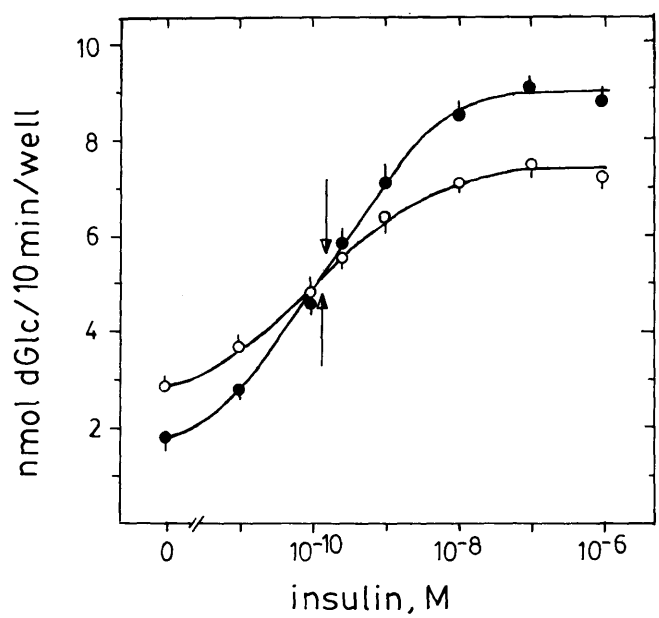

FIGURE 6. Long-term effect of low pH on the insulin-sensitive dGlc uptake. dGlc uptake (10 min, 37°C) was measured at physiologic pH after 48 h of incubation at pH 6.90 (○) or pH 7.4 (●). The arrows indicate the half-maximal effective concentration of insulin.

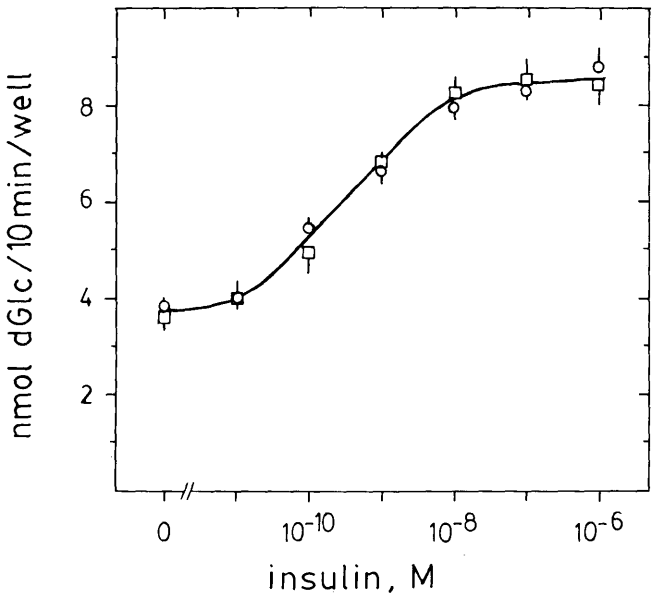

FIGURE 7. Long-term effect of beta-hydroxybutyrate on the insulin-stimulated dGlc uptake. dGlc uptake (10 min, 37°C, pH 7.40) was measured in the absence of beta-hydroxybutyrate after 48 h of incubation in the presence (□) or absence (○) of the compound in a medium of pH 6.90.

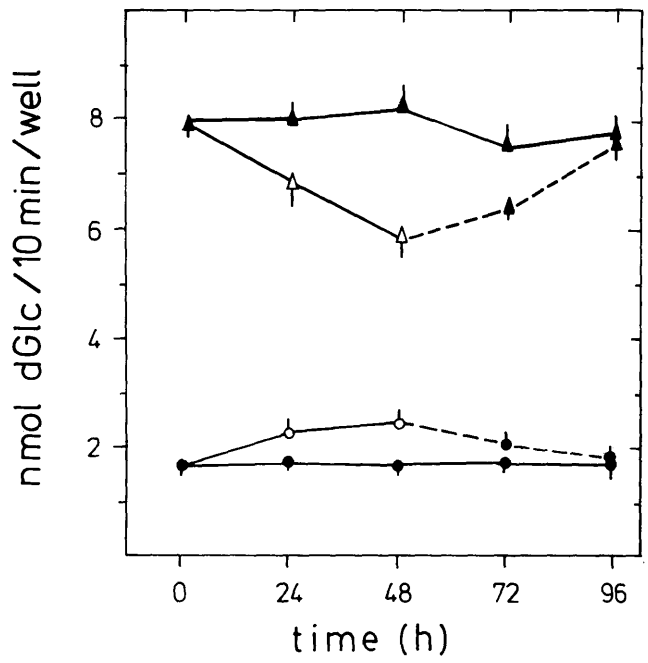

FIGURE 8. Time course and reversibility of the long-term effect of low pH on the insulin-sensitive dGlc uptake. Fat cells were cultured at *pH 6.90* (○,△) *and pH 7.40* (●,▲). *At selected times* the basal (○,●) and maximal insulin-stimulated (10^{-6} M) (△,▲) dGlc uptake (10 min, 37°C) were determined.

binding to the cultured fat cells was decreased by up to 50%, primarily due to a reduction in the rate of association of insulin to its binding sites. Note that a reduced insulin binding at low pH due to a decrease in association rate has also been found in most [10,23,24] but not all[21] studies of rat and human adipocytes. In cultured IM-9 human lymphocytes, the rate of dissociation is accelerated at low pH,[12] suggesting cell-type differences. The alteration in the insulin-receptor interaction observed may result from an effect of pH on the receptor charge as well as on the conformation of the insulin molecule.[7] The importance of the state of the receptor is emphasized by studies on cells of patients with leprechaunism and of patients with a type A syndrome of insulin resistance and acanthosis nigricans. These cells, which have abnormal insulin binding properties, hardly show any pH dependence of the insulin-receptor interaction.[25] Recently, Harmon et al.[26,27] proposed a mechanism of receptor affinity regulation based on the interaction of two functional components of the insulin receptor: a binding component and an affinity regulator. The degree of association of the components regulating receptor affinity was influenced by insulin binding and pH. In rat liver membranes, lowering of pH induced a reversible increase in the interaction of the two components with a concomitant decrease in receptor affinity.[28] It is possible that the effect of low pH on insulin binding in the cultured fat cells is mediated by the same mechanism.

The basal hexose uptake and the insulin responsiveness were only slightly decreased at low pH, indicating that in the "acute" stage postbinding alterations are not a predominant feature. This moderate effect of low pH on the hexose uptake in the cultured 3T3 fat cells is consistent with observations in isolated rat adipocytes,[9] although more drastic effects of pH on hexose uptake have been reported.[10] Low pH did induce a marked decrease in insulin sensitivity. At submaximal insulin concentrations, hexose uptake was decreased by up to 60%. This effect can, at least partially, be explained by the decrease in receptor affinity and perhaps by the postbinding alterations responsible for the slight decrease in the maximal insulin effect (insulin responsiveness). Taken together, the data on the acute effect of low pH on the insulin effectiveness in cultured fat cells suggest that in vivo, during ketoacidosis, low pH contributes to insulin resistance, primarily by altering the insulin-receptor interaction.

In vivo, low pH is often accompanied by increased concentrations of ketone bodies in the plasma (ketoacidosis), which might contribute to the insulin resistance as well. In the cultured fat cells, "acute" exposure to beta-hydroxybutyrate induced a pH-dependent increase in insulin binding and insulin sensitivity. The basal hexose uptake and the insulin responsiveness were not affected. The acute effects of DL-beta-hydroxybutyrate on insulin binding and sensitivity were dose dependent with an ED_{50} of 10 mM. In vivo, during ketoacidosis, concentrations of the natural isomer D-3-hydroxybutyrate between 5 and 10 mM are not uncommon,

TABLE 1
Transport kinetics of dGlc uptake

	pH 6.90	pH 7.40	P-value
K_m (mM)	2.2 ± 0.2	2.0 ± 0.1	NS
V_{max} (nmol/min/well)	5.7 ± 0.1	4.2 ± 0.1	<0.005

Uptake (2 min) of various concentrations of dGlc (0.05–2 mM) by cells cultured at pH 6.9 and pH 7.4 for 48 h. After subtraction of the uptake in the presence of cytochalasin B (0.1 mM), which was <2% of the total uptake, the data were analyzed according to Lineweaver-Burke. The data expressed are the mean ± SEM of five separate experiments.

suggesting that our data may have clinical relevance. In other cell types, the effects of ketoacids on the insulin effectiveness are controversial. In IM-9 lymphocytes, an insulin-insensitive cell line, Misbin et al.[8] and Merimee et al.[13] reported a pH-dependent, binding-enhancing effect of ketoacids. By contrast, Olefsky et al.[14] were not able to show any effect of ketoacids on IM-9 lymphocytes. In cultured human fibroblasts, 3-hydroxybutyrate has been reported to enhance insulin binding, but this effect was not accompanied by an increase in insulin sensitivity,[11] which might be related to the fact that fibroblasts are not primary target cells of insulin. In adipocytes, effects of ketoacids on insulin binding have not been reported before, but ketoacids have been shown to enhance the insulin-sensitive glucose uptake.[29] The mechanism(s) by which ketoacids enhance the insulin binding and insulin response in cultured fat cells is unclear. The effect of low pH is partially counteracted. Yet, the added solution of beta-hydroxybutyrate was buffered and did not affect the pH of the incubation buffer. Ionic strength, known to enhance insulin binding,[28,29] did not seem to be involved either. The observation that the binding-enhancing effect is more pronounced at low pH than at physiologic pH and nearly absent at optimum pH suggests a relation to the mechanism by which low pH regulates receptor affinity. These data on the acute effects of ketoacids on the insulin effectiveness suggest that, in vivo during ketoacidosis, ketoacids have an antagonizing rather than a synergistic effect on the insulin resistance-inducing effect of low pH.

In vivo ketoacidosis may last for several days. Nevertheless, the effects of prolonged exposure of cells to a low pH and of ketoacids on insulin effectiveness have not been studied before. In cultured fat cells, restoration of the physiologic pH (pH 7.4) after 48 h of culture at pH 6.90 instantly normalized insulin binding and insulin sensitivity. Apparently, there is no long-term effect of low pH on insulin receptor regulation. In contrast, the insulin response was decreased up to 40% after 48-h incubation at low pH. This was a result of an increase in basal hexose uptake and a decrease in insulin responsiveness. This long-term effect of low pH was maximal after 48 h of incubation and was only slowly reversible (48 h). The increase in basal hexose uptake was due to an increase in the apparent V_{max} of the transport system, i.e., to an increase in the number or activity of the hexose transporters. The mechanism by which prolonged exposure to low pH affects hexose transporter regulation remains to be solved. The increase in basal hexose uptake together with the absence of alterations in insulin binding and insulin sensitivity suggest that the process is independent of the effect of pH on the regulation of insulin receptor binding. That the acute and long-term effect of low pH are probably mediated by different mechanisms is further emphasized by the observation that incubation at low pH was not influenced by ketoacids, in contrast to the counteracting effect of ketoacids on the acute effect of low pH. As a matter of fact, on a long-term basis, ketoacids did not affect the insulin binding or the insulin-sensitive hexose uptake. Our in vitro observations of the long-term effects of low pH suggest that in vivo, depending on duration of ketoacidosis, postreceptor alterations are induced. These might contribute to the insulin resistance in addition to the acute effect of low pH on insulin binding. Studies on rats suggest the existence of postreceptor alterations during acidosis in vivo.[3] Ketoacids probably do not contribute to the insulin resistance accompanying ketoacidosis in vivo.

In summary, our results indicate that, in cultured fat cells, acute exposure to low pH induces insulin resistance primarily by rapidly reversible alterations in the insulin-receptor interaction, which can be counteracted by ketoacids. At prolonged exposure to low pH, postbinding alterations become predominant, which are only slowly reversible and not affected by ketoacids. These in vitro observations may explain the insulin resistance often present during ketoacidosis in vivo and emphasize the importance of the duration of ketoacidosis in the interpretation of in vivo studies. If, indeed, the in vitro observations are applicable in vivo, the slow reversibility of the postreceptor alterations implicates that in vivo it will take some days before restoration of the physiologic pH results in normalization of the insulin response.

ACKNOWLEDGMENTS

We thank Wies Dijkstra-van der Star for excellent technical assistance with the cell culture, and Carina van den Haak for typing the manuscript.

This work was supported by the Foundation of Medical Research FUNGO, which is subsidized by the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

[1] Walker, B. G., Phear, D. N., Martin, F. I. R., and Baird, C. W.: Inhibition of insulin by acidosis. Lancet 1963; 2:964–65.

[2] Roth, J., Kahn, C. R., Lesniak, M. A., Gordon, P., De Meyts, P., Megyesi, K., Neville, D. M., Jr., Gavin, J. R., III, Soll, A. H., Freychet, P., Goldfine, I. D., Bar, R. S., and Archer, J. A.: Receptor for insulin, NSILA-S and growth hormone: applications to disease states in man. Recent Prog. Horm. Res. 1975; 31:95–140.

[3] Cuthbert, C., and Alberti, K. G. M. M.: Acidemia and insulin resistance in the diabetic ketoacidotic rat. Metabolism 1978; 27:1903–16.

[4] Barrett, E. J., DeFronzo, R. A., Bevilacqua, S., and Ferrannini, E.: Insulin resistance in diabetes ketoacidosis. Diabetes 1982; 31:923–28.

[5] Whittaker, J., Cuthbert, C., Hammond, V., and Alberti, K. G. M. M.: Impaired insulin binding to isolated adipocytes in experimental diabetic ketoacidosis. Diabetologia 1981; 21:563–68.

[6] Ginsberg, H. N.: Investigation of insulin resistance during diabetic ketoacidosis: role counterregulatory substances and effect of insulin therapy. Metabolism 1977; 26:1135–46.

[7] Waelbroeck, M.: The pH dependency of insulin binding. J. Biol. Chem. 1982; 257:8284–91.

[8] Misbin, R. I., Pulkkinen, A. J., Lofton, S. A., and Merimee, T. J.: Ketoacids and the insulin receptor. Diabetes 1978; 27:539–42.

[9] Sonne, O., Gliemann, J., and Linde, S.: Effect of pH on binding kinetics and biological effect of insulin in rat adipocytes. J. Biol. Chem. 1981; 256:6250–54.

[10] Kikuchi, K., and Larner, J.: Independent control of selected insulin-sensitive cell membrane and intracellular functions—the linkage of cell membrane and intracellular events controlled by insulin. Mol. Cell. Biochem. 1981; 37:109–15.

[11] Hidaka, M., Howard, B. V., Ishibashi, F., Kosmakos, F. C., Craig, J. W., Bennett, P. H., and Larner, J.: Effect of pH and 3-hydroxybutyrate on insulin binding and action in cultured human fibroblasts. Diabetes 1981; 30:402–406.

[12] De Meyts, P., Bianco, A. R., and Roth, J.: Site-site interactions among insulin receptors. J. Biol. Chem. 1976; 251:1877–88.

[13] Merimee, T. J., Pulkkinen, A. J., and Lofton, S. A.: Increased insulin binding by lymphocyte receptors induced by β-OH butyrate. J. Clin. Endocrinol. Metab. 1976; 43:1190–92.

[14] Olefsky, J. M.: Mechanisms of fasting-induced increase in insulin binding to rat adipocytes. J. Clin. Invest. 1978; 61:329–38.

[15] Green, H., and Kehinde, O.: Sublines of mouse 3T3 cells that accumulate lipid. Cell 1974; 1:113–16.

[16] Rubin, C. S., Hirsch, A., Fung, C., and Rosen, O. M.: Development of hormone receptors and hormonal responsiveness in vitro. J. Biol. Chem. 1978; 253:7570–78.

[17] Reed, B. C., and Lane, M. D.: Insulin receptor synthesis and turnover in differentiating 3T3-L_1 preadipocytes. Proc. Natl. Acad. Sci. USA 1980; 77:285–89.

[18] Sedmak, J. J., and Grossberg, S. E.: A rapid, sensitive and versatile assay for protein using Coomassie brilliant blue G 250. Anal. Biochem. 1977; 79:544–53.

[19] Gorden, P., Carpentier, J. L., Freychet, P., and Orci, L.: Internalization of polypeptide hormones. Diabetologia 1980; 18:263–74.

[20] Häring, H. U., Biermann, E., and Kemmler, W.: Coupling of insulin binding and insulin action on glucose transport in fat cells. Am. J. Physiol. 1981; 240:E556–65.

[21] Marshall, S., Podlecki, D. A., and Olefsky, J. M.: Low pH accelerates dissociation of receptor-bound insulin. Endocrinology 1983; 113:37–42.

[22] Marshall, S., and Olefsky, J. M.: Characterization of insulin-induced receptor loss and evidence for internalization of the insulin receptor. Diabetes 1981; 30:746–53.

[23] Scatchard, G.: The attractions of protein for small molecules and ions. Ann. NY Acad. Sci. 1949; 51:660–75.

[24] Pedersen, O.: Hexose transport in human adipocytes: factors influencing the response to insulin and kinetics of methylglucose and glucose transport. Diabetologia 1981; 20:630–35.

[25] Podskalny, J. M., and Kahn, C. R.: Cell culture studies on patients with extreme insulin resistance. I. Receptor defects on cultured fibroblasts. J. Clin. Endocrinol. Metab. 1982; 54:261–68.

[26] Harmon, J. T., Kahn, C. R., Kempner, E. S., and Schlegel, W.: Characterization of the insulin receptor in its membrane environment by radiation inactivation. J. Biol. Chem. 1980; 255:3412–19.

[27] Harmon, J. T., Kempner, E. S., and Kahn, C. R.: Demonstration by radiation inactivation that insulin alters the structure of the insulin receptor in rat liver membranes. J. Biol. Chem. 1981; 256:7719–22.

[28] Harmon, J. T., Hedo, J. A., and Kahn, C. R.: Characterization of a membrane regulator of insulin receptor affinity. J. Biol. Chem. 1983; 258:6875–81.

[29] Cuatrecasas, P.: Properties of the insulin receptor of isolated fat cell membranes. J. Biol. Chem. 1971; 246:7265–74.