Insulin-induced Desensitization at the Receptor and Postreceptor Level in Mitogen-activated Human T-Lymphocytes

LOUIS ERCOLANI, HSIN LEE LIN, AND BARRY H. GINSBERG

SUMMARY

Human T-lymphocytes activated by phytohemagglutin acquire insulin receptors in culture. Saturation analysis of insulin-binding activity in the presence of competing ligand revealed curvilinear Scatchard plots. Insulin receptors were not regulated by insulin before mitogen activation and culture of T-lymphocytes. However, insulin-induced downregulation of insulin receptors was: (1) demonstrable in receptor-positive cells, (2) dependent on insulin concentration, (3) temporally unrelated to insulin internalization, and (4) prevented by culture at 4°C but not by cycloheximide at 37°C. Recovery of insulin receptors required further culture of cells in media depleted of insulin for 24 h. Scatchard analysis revealed loss of receptor number without changes in receptor affinity.

Insulin-induced increases in glucose transport and oxidation were demonstrable in receptor-positive cells but not in receptor-negative cells. However, these effects were extremely time-dependent. After a 2-h exposure of cells to 10^{-8} M insulin, increases in glucose transport were no longer demonstrable. Elution of bound insulin from these cells followed by re-exposure to insulin depressed glucose transport in them. Recovery from this hyporesponsive, desensitized state required a 6-h culture in insulin-depleted media. Glucose oxidation of desensitized cells could be stimulated by spermine but not by insulin. These studies demonstrate the activated human T-lymphocyte is an insulin-sensitive tissue that is capable of limiting its physiologic response to insulin by receptor- and postreceptor-mediated mechanisms. DIABETES 1985; 34:931–37.

The mechanism of insulin action in normal and diabetic target tissues remains largely unknown. A readily available insulin-sensitive human tissue source would be clearly advantageous for studying insulin receptor-ligand interactions that are coupled to physiologic responses.

Freshly isolated human T-lymphocytes obtained from peripheral blood are a readily available tissue source, but do not bear insulin receptors. However, upon activation by lectin or alloantigen, insulin receptors can be detected on this cell type during in vitro culture well in advance of cell division.[1–3] Insulin-binding activity appears to be linked to a mitogen- or antigen-mediated cascade of biochemical events within T-lymphocytes that ultimately leads to receptor synthesis and subsequent plasma membrane insertion.[4,5] Recent studies from this laboratory have demonstrated the T-lymphocyte insulin receptor displays "classical" binding properties and requires specific saccharidic moieties for binding its ligand, similar to other insulin-sensitive tissues.[6]

Adipocytes, which are classic targets for insulin action, are capable of increasing their intermediary metabolism in response to insulin.[7] However, these cells are also capable of reversibly limiting their response to hyperinsulin states by receptor- and postreceptor-mediated mechanisms.[8,9] Receptor-mediated mechanisms include downregulation, a time- and temperature-dependent process that specifically reduces the number of receptors available for ligand binding. Postreceptor mechanisms include desensitization, a process that limits the normal physiologic increases in a cell's intermediary metabolism seen after ligand binding to receptor.[10]

In the present study, we examine (1) the ability of human T-lymphocytes activated by phytohemagglutin (PHA) to respond to insulin-mediated increases in their intermediary metabolism, and (2) whether normal regulatory mechanisms limit these cells' physiologic response to high concentrations of insulin. Our findings suggest that the activated human T-lymphocyte is remarkably similar to less readily available insulin-sensitive tissues, such as adipocytes and hepatocytes, whose response to the polypeptide hormone insulin is principally governed by the concentration of hormone and the presence of a specific cell surface receptor for hormone binding.

From the Veterans Administration Medical Center, Diabetes-Endocrinology Research Center, and the Departments of Medicine and Biochemistry, University of Iowa, Iowa City, Iowa.

Address reprint requests to Louis Ercolani, M.D., Howard Hughes Medical Institute, Massachusetts General Hospital, Blossom Street, Wellman 3, Boston, Massachusetts 02114.

Received for publication 5 December 1983 and in revised form 1 March 1985.

MATERIALS AND METHODS

Materials. Ficoll-Hypaque was obtained from Pharmacia Fine Chemicals, Piscataway, New Jersey; spermine, cycloheximide, and morpholinopropane sulfonic acid (MOPS) were from Sigma Chemical Co., St. Louis, Missouri; 1640 RPMI media (RPMI), phosphate-buffered saline (PBS), and fetal bovine serum were from Grand Island Biological Co., Grand Island, New York; Barbitone buffer was from Oxoid Ltd., United Kingdom; single-peak porcine insulin was a kind gift of Dr. Ronald Chance, Eli Lilly and Company, Indianapolis, Indiana; nylon wool and dupanol were gifts from the Dupont Nemours Co., Wilmington, Delaware; bovine thrombin was from Parke Davis and Co., Detroit, Michigan; PHA from phaseolus vulgaris (purified HA16) was from Wellcome Research Laboratories, Beckenham, United Kingdom; and [^{3}H]-2-deoxyglucose (37.3 Ci/mmol), [^{14}C]-L-glucose (47 mCi/mmol), and [^{14}C(u)]-D-glucose (250 Ci/mmol) were from New England Nuclear, Boston, Massachusetts.

Peripheral blood mononuclear cells (PBM) were isolated from healthy, normal subjects ages 21–34 yr by centrifugation of heparinized, phosphate-buffered-saline–diluted blood samples over a Ficoll-Hypaque density gradient.[11]

T-lymphocytes were prepared by sequential removal of platelets and phagocytic cells from PBM with thrombin as previously described[5] followed by passage of remaining cells over nylon wool columns prewetted with Barbitone buffer supplemented with 5% fetal bovine serum. Cells recovered were 99% T-lymphocytes as assessed by sheep erythrocyte rosetting. Less than 1% of cells were monocytes as assessed by esterase staining.[12]

Cellular activation. Matched T-lymphocytes at 1×10^6 cells/ml in RPMI containing 10% fetal bovine serum, 25 mmol Hepes, 100 U penicillin, and 100 μg streptomycin were distributed in flat-bottom flasks (Corning 25115). PHA 2 μg/ml (a concentration previously shown to produce maximal blastogenesis as determined by dose-response curves under our experimental conditions) was added to cultures, which were subsequently incubated in a 95% humidified air/5% CO_2 atmosphere. Typical mitogenic responses assessed by radioactive uptake of [^{3}H]-thymidine (6.7 Ci/mmol/L) from 56 to 72 h of culture varied between individuals (94,000–125,000 cpm/0.2 $\times$ 10^6 cells). Mitogenic proliferation was abolished at lower cell density culture conditions (10^5 cells or less/ml).

Radioactive ligand preparation. A_{14}-mono-[^{125}I]-iodoinsulin was prepared as previously described.[13] Iodide and degraded constituents were removed by high-pressure liquid chromatography. The specific activity of [^{125}I]-iodoinsulin preparation varied from 200 to 400 Ci/g.

Insulin binding assay was performed as previously described.[5] Briefly, cells were washed three times in HL buffer (100 mM Hepes, 120 mM NaCl, 25 mM Na acetate, 10 mM glucose, 2.4 mM KCl, 0.81 mM $MgSO_4$, 0.81 mM EDTA, and 10 mg/ml bovine serum albumin) and adjusted to 0.5 ml at 1.5–3.0 $\times$ 10^7 cells/cc in each assay (depending on cell yield from individual donors) to which 20 pM [^{125}I]-iodoinsulin and 0–10 μg/ml unlabeled insulin was added. Cells were then incubated for 2 h at 15°C. Duplicate 0.2 ml aliquots of incubated cells were then layered over an equal volume of HL buffer and sedimented at 10,000 $\times$ *g* for 1 min. The supernatant was aspirated. The portion of the tube containing the pellet was cut. Cell-associated radioactivity was determined by counting cell-containing tube tips in a Beckman Gamma 300 counter (Beckman Instruments, Fullerton, California) at 78% efficiency for 10 min. Nonspecific binding was defined as the amount of [^{125}I]-iodoinsulin bound to cells in the presence of 10^{-5} M unlabeled insulin and was approximately 400 cpm or <1% of the tracer added. Residual radioactivity in tips without cells was <0.08% of the total tracer added. Degradation of the iodoinsulin was always <10% as determined by trichloracetic acid (TCA) precipitability. Binding data were analyzed by a computer program (DERC-2) written for a Hewlett-Packard 2000 computer that "curve fits" the competition curve to a fourth-order polynomial. The program generated a Scatchard plot of the fitted curve and analyzed the binding data of both the De Meyts[14] and two-site models.[15]

Insulin receptor downregulation. PHA-treated T-lymphocytes were cultured for 72 h. Single-peak porcine insulin (0–10^{-7} M) was then added to cultures. At varying times thereafter, bound insulin was dissociated from its receptor by three separate washes of cells in PBS for 15 min at 30°C followed by a final wash of cells with PBS. This technique removes surface-bound insulin without affecting receptor properties.[16] Insulin-binding activity on matched cells cultured with or without insulin was then determined.

2-Deoxyglucose transport. After dissociation of insulin from receptors, insulin-binding assays were used to confirm the presence of insulin receptors on T-lymphocytes treated with or without various concentrations of insulin. Measurement of glucose transport was determined by modification of the method of Helderman.[17] One-tenth milliliter of cells was adjusted to 2.5 $\times$ 10^7/ml in transport buffer at pH 7.4 containing 90 mM NaCl, 8 mM KCl, 0.4 mM $CaCl_2 \cdot 6H_2O$, 0.4 mM $MgSO_4 \cdot 7H_2O$, 50 mM MOPS, and 2 mM NaH_2PO_4 in 12 $\times$ 75-mm glass tubes containing 50 μl of a tritiated to unlabeled 2-deoxyglucose mixture (1:50). The reaction was allowed to proceed at 26°C in a shaking water bath and stopped at various times by placing 150 μl of the reaction mixture on a bed of phthalate oils in plastic microfuge tubes (18:4 ratio of n-butyl phthalate to corn oil), then centrifuging at 10,000 $\times$ *g* in a Beckman microfuge. The cell pellet was obtained by cutting the tip of the centrifuge tube to which 50 μl of protosol (New England Nuclear) was added. After dissolution of the cells, the tip was added to 3 ml of scintillation cocktail (toluene containing 5.7 mg POPOP and 57 mg PPO). Radioactivity of the vials was determined by counting in a Beckman Scintillation Counter. The separation method allowed recovery of >98% of cells with <0.01% contamination of the oil by free isotope. The effect of insulin on the flux rate of 2-deoxyglucose of these cells was determined by preincubation of 100 μl of cells with single-peak porcine insulin 10 nM or transport buffer for 10 min at 37°C. The flux rate was determined from the slope of the rapid phase of the transport time course when the reaction was stopped at 0, 1, 3, and 5 min. Diffusive transport was determined by addition of 0.1 μCi [^{14}C]-L-glucose to the above reaction mixture. Specific sodium-dependent transport of glucose was obtained by subtracting transport obtained from diffusion alone.

Carbon dioxide production from glucose. The production of CO_2 from glucose by PHA-activated human T-lymphocytes was measured by slight modification of the method of Rodbell.[7] Insulin receptor-positive T-lymphocytes were adjusted

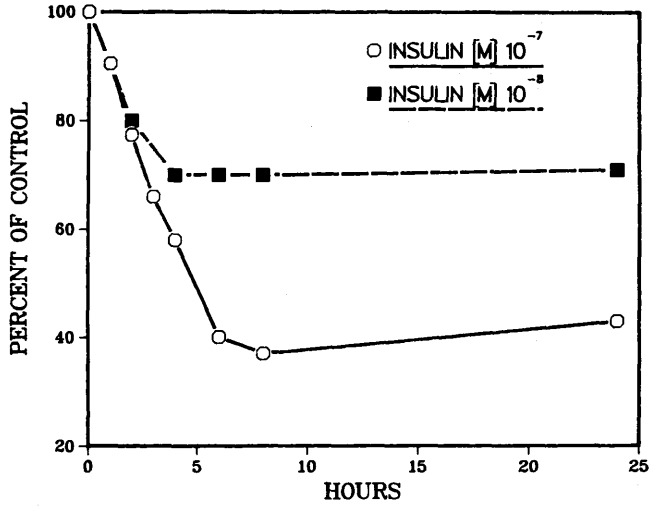

FIGURE 1. Insulin-induced downregulation of insulin-binding activity during culture of activated T-lymphoblasts from a single individual. PHA-activated T-lymphocytes that had acquired [^{125}I]-iodoinsulin binding activity after 3 days of culture were treated with or without insulin (10^{-7} M or 10^{-8} M). After elution of bound insulin from cells as described in the text, insulin-binding activity was then determined in these cells over a 24-h period. Data are expressed as a percentage of control insulin-binding activity.

to 6 × 10^6 cells/ml of oxidation buffer (NaCl, 128 mM; $CaCl_2$, 1.4 mM; $MgSO_4$, 1.4 mM; KCl, 5.2 mM; Na_2HPO_4, 10 mM; D-glucose, 4 mM; and 3% bovine serum albumin), adjusted to pH 7.4, and placed in plastic 17 × 180-mm tubes. To these tubes, 0.1 μCi of [^{14}C]-1-D-glucose was added followed by sealing with rubber serum stoppers fitted with hanging plastic wells to which Whatman no. 1 paper strips were attached. The cells were then incubated at 37°C for 2 h in a 100% O_2 atmosphere. At the end of the incubation period, 0.2 ml Hyamine-10X (Sigma) was injected into the Whatman paper immediately followed by injection of the cells with 0.2 ml of

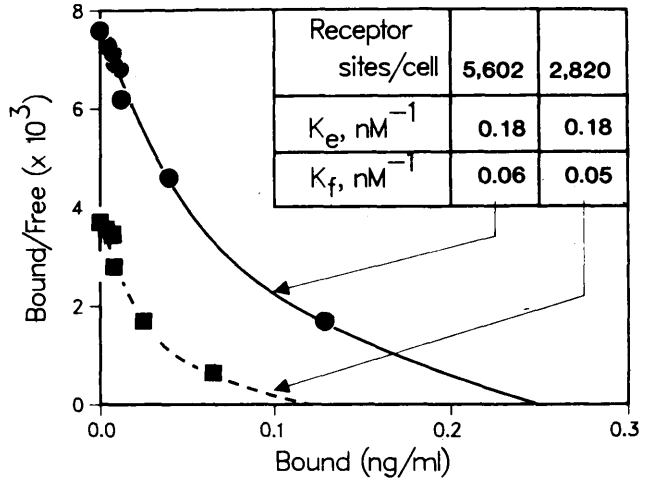

FIGURE 2. Scatchard plot of A_{14}-mono-[^{125}I]-iodoinsulin binding of PHA-activated human T-lymphocytes from a single individual cultured for 3 days, then treated with or without 10^{-7} M insulin for an additional 24 h of culture. The data were analyzed and fitted by computer as described in the text. Data are presented as a receptor single-site model: K_e = affinity constant for unoccupied receptors, K_f = affinity constant for occupied receptors. Control cells, ●——●; insulin-treated cells, ■----■.

1 N H_2SO_4 to stop the cellular production of CO_2. The reaction was allowed to proceed for an additional 15 min. Paper strips were then removed and immersed in 10 ml of scintillation cocktail. Radioactivity was determined by counting vials in a Beckman Scintillation Counter. CO_2 production was estimated by multiplying counts per minute trapped by the specific activity of the added radiolabeled glucose determined for each run.

Statistical analysis. Student's *t*-test was used to analyze paired data.

RESULTS

Effects of insulin on insulin-binding activity of T-lymphoblasts. Insulin has been reported to inversely affect the number of insulin receptors on a variety of tissues.[8,9,18,19] Freshly isolated peripheral blood mononuclear cells (PBM) were therefore cultured with or without daily insulin (10^{-7} M) for 3 days. These cells were then washed in PBS to remove insulin; depleted of monocytes, phagocytes, and B-lymphocytes; treated with PHA; cultured for an additional 3 days; and analyzed for insulin-binding activity of developed T-lymphoblasts. No differences in insulin-binding activity could be demonstrated in cells initially pretreated with insulin as compared with cohorts that were not.

In subsequent experiments, we questioned whether insulin-mediated reductions in insulin binding activity required the presence of cell surface insulin receptors. PHA-treated T-lymphocytes that had acquired insulin receptors during in vitro culture for 3 days were incubated with varying concentrations of insulin for an additional 24 h at 37°C. These cells were then washed to remove bound insulin and were examined for insulin-binding activity. Insulin-binding activity of cells treated with 10^{-9} M, 10^{-8} M, and 10^{-7} M insulin were reduced by 23 ± 6, 27 ± 4.8, and 56 ± 5.2%, respectively, when compared with cohort controls in nine experiments. As seen in Figure 1, reduction in insulin-binding activity of insulin-treated cells was maximal by 6 h for both 10^{-7} M and 10^{-8} M insulin. Removal of insulin from cells after 2 h of culture did not impede maximal receptor loss. Insulin-binding activity of cells treated with or without 10^{-7} M insulin, when analyzed by the method of Scatchard, revealed curvilinear plots demonstrating loss of receptor numbers without changes in affinity, as seen in Figure 2. Recovery of insulin receptors to

TABLE 1
Effects of temperature and protein synthesis inhibition on insulin-induced downregulation of T-lymphoblast insulin-binding activity (IBA)*

Treatment	IBA as mean percent of control ± SEM†
4°C	99 ± 2.0
37°C	52 ± 6.8
Cycloheximide at 37°C	53 ± 7.4

*Matched, PHA-activated T-lymphocytes from three individuals were cultured with or without 10^{-7} M insulin for 24 h. At the beginning of these experiments, insulin-exposed cells were cultured at 37°C with or without 25 μg/ml cycloheximide or cultured at 4°C. Insulin-binding activity was then determined at the end of culture as described in the text. Protein synthesis was inhibited in cycloheximide-treated cells by 92%; however, cycloheximide treatment alone decreased IBA of these by only 12%.
†Control represents IBA of PHA-activated T-lymphocytes after 24 h of culture at 37°C without insulin added.

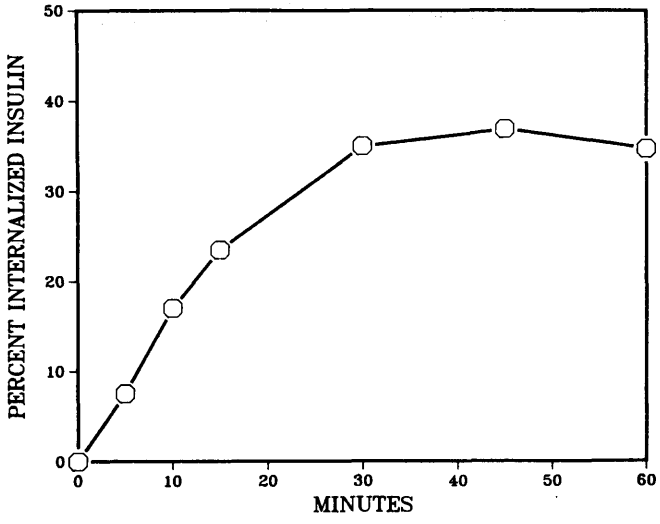

FIGURE 3. Internalization of insulin by T-lymphoblasts. PHA-activated T-lymphocytes were incubated with A_{14}-mono-[^{125}I]-iodoinsulin for 24 h at 4°C to saturate all binding sites. Cells were subsequently washed to remove unbound iodoinsulin, then warmed to 26°C. After varying times aliquots of cells were removed, trypsinized to remove cell surface insulin receptors, washed, and then solubilized with NaOH. Radioactivity of TCA-precipitable material of solubilized cells (intact insulin) was then counted by scintillation spectroscopy. Data are expressed as percent of internalized intact iodoinsulin after subtracting counts obtained at time 0.

normal levels required an additional 24 h of incubation in media depleted of insulin. To determine if insulin receptor loss was a temperature-dependent process or required the synthesis of a protein for insulin receptor degradation, cells were treated with 10^{-7} M insulin at 4°C and at 37°C in the presence of 25 μg/ml of cycloheximide. As seen in Table 1, treatment of cells with insulin at 4°C did not result in loss of insulin-binding activity; however, receptor loss was not prevented by treatment with cycloheximide when compared with untreated cohorts. These data suggested that while insulin receptor loss was mediated, in part, by active metabolic processes, the production of new degradative proteins was not required.

As insulin internalization is mediated by receptor-mediated endocytotic processes in adipocytes,[20] we questioned whether receptor loss in T-lymphocytes could be due to internalization of receptor-ligand complexes. For these experiments, insulin binding sites of T-lymphoblasts were saturated by exposure to [^{125}I]-iodoinsulin for 24 h at 4°C. These cells were then washed in PBS at 4°C to remove nonbound insulin and then warmed to 26°C. After varying times, the cells were immersed in 4°C PBS and subjected to surface trypsinization followed by solubilization and counting of intact internalized insulin. As seen in Figure 3, insulin was rapidly internalized: 36% of intact insulin was found within these cells by 30 min. As significant receptor loss could not be demonstrated within this time period, these data suggested rapid replacement of insulin receptors at the cell surface by newly synthesized receptors or receptors that had been endocytosed and recycled to the cell surface.

Effect of insulin on glucose transport and glucose oxidation of T-lymphocytes. T-lymphocytes that were not PHA treated did not have demonstrable insulin-binding activity. Basal flux rates for 2-deoxyglucose transport in these cells were found to be identical to those of L-glucose transport (1–3 fmol/10^6 cells · min) and were unaffected by high concentrations of insulin (10^{-7} M). These data suggested glucose transport occurred principally by diffusive processes in these cells. Upon PHA activation, basal flux rates uniformly increased to 7–9 fmol/10^6 cells · min in nine experiments ($P < 0.005$) whereas diffusive transport remained unchanged. Treatment of PHA-activated T-lymphocytes that had acquired insulin receptors during culture with varying concentrations of insulin uniformly increased glucose transport in comparison with non-insulin-treated cohorts over a physiologic range of insulin concentrations maximizing at 10^{-9} M, as seen in Figure 4. To determine whether PHA-activated T-lymphocytes could be desensitized to high concentrations of insulin, cells were treated with or without 10^{-8} M insulin for 2 h at 37°C followed by wash in PBS to elute bound insulin from its receptor. Both controls and treated cells were then exposed to 10^{-8} M insulin and examined for glucose transport. As seen in Figure 5, in 15 experiments, naive cells exposed to insulin for the first time appropriately increased their glucose transport over basal rates ($P < 0.01$). However, cells reexposed to insulin were desensitized and demonstrated a somewhat decreased glucose transport when compared with non-insulin-treated cohorts ($P < 0.001$). Figure 6 demonstrates that recovery of desensitized cells required 6 h of culture in the absence of insulin. To determine whether this defect in glucose transport was at an early postreceptor site in the pathway of insulin action, naive cells and desensitized cells were examined for glucose oxidation in the presence of spermine, an agent known to act on glucose metabolism independently of the insulin receptor.[21,22] As seen in Table 2, naive cells' glucose oxidation was maximized by either 10^{-8} M insulin or 10 μM spermine. Desensitized cells' glucose oxidation could not be stimulated by insulin but could be maximally stimulated by spermine. These data suggested desensitization to insulin was occurring at an early postreceptor step.

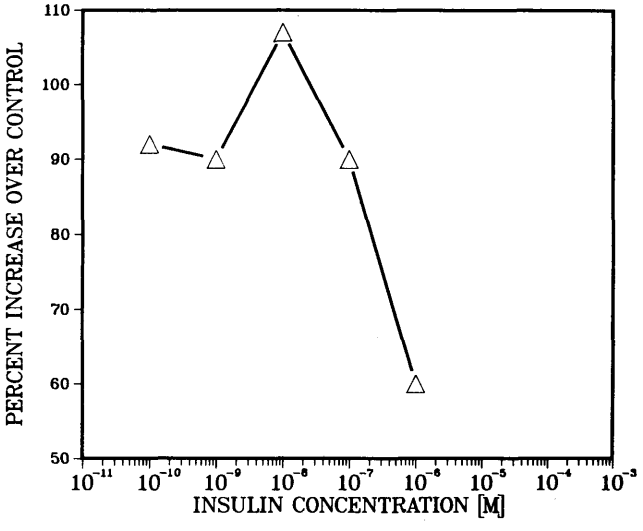

FIGURE 4. Insulin-induced increases in activated T-lymphoblast glucose transport. Activated T-lymphocytes from a single individual were treated with or without varying concentrations of insulin as described in the text. Data are plotted as percentage increases over control cells after subtracting transport of glucose attributable to diffusion alone.

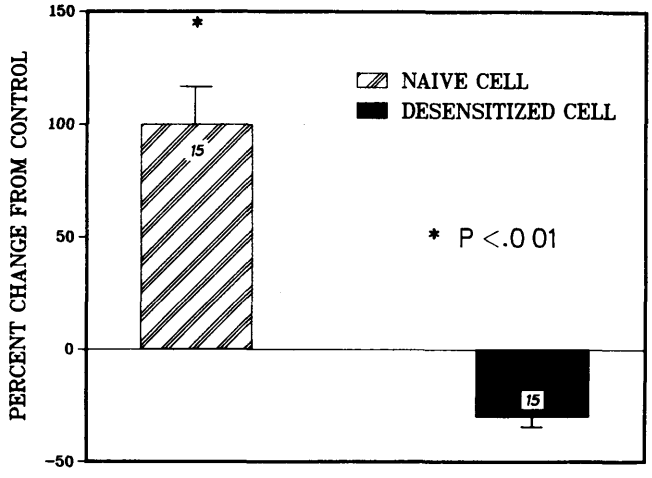

FIGURE 5. Insulin-induced desensitization in activated T-lymphoblast glucose transport. As described in the text, matched, activated T-lymphocytes from 15 individuals were examined for basal glucose transport, glucose transport in the presence of 10^{-8} M insulin (naive cells), and glucose transport after cells had been exposed to insulin 10^{-8} M for 2 h (desensitized), washed to remove unbound insulin, and then reexposed to insulin 10^{-8} M. Data from matched cells are expressed as mean percent change (± SEM) from basal glucose transport after subtracting glucose transport attributable to diffusion alone.

DISCUSSION

These studies demonstrate that the intermediary metabolism of peripheral blood human T-lymphocytes is increased by physiologic concentrations of insulin only after these cells acquire insulin receptors. Our findings are similar to earlier observations of Helderman using T-lymphocytes derived from splenic tissues in rodents.[17] Glucose transport in nonactivated T-lymphocytes occurred principally by diffusive processes. On activation by phytohemagglutinin, basal rates of diffusive glucose transport remained unchanged, whereas sodium-dependent processes increased glucose transport in T-lymphocytes. Similar findings have been reported for T-lymphocytes using a variety of mitogenic agents.[23] On acquisition of insulin-binding activity, insulin-mediated increases in glucose transport and oxidative metabolism could be demonstrated.

Previous studies from this laboratory confirmed that human T-lymphocytes developed in serum-free medium were capable of insulin-mediated downregulation of their insulin receptors.[6] The present study demonstrates that human T-lymphocytes developed in serum-containing medium were similarly capable of regulating their surface insulin receptors in response to insulin. As in other tissues such as adipocytes, downregulation appears to be dependent on active cellular metabolism[24] but differs from another cell type, the IM-9 lymphocyte, insofar as newly synthesized proteins capable of receptor degradation are not required to mediate this process.[16] As the IM-9 lymphocyte's response to insulin is defective, these differences may be attributable to the IM-9's neoplastic origin.

Treatment of nonfractionated peripheral blood mononuclear cells (which contain a mixture of cells including T-lymphocytes, B-lymphocytes, monocytes, and granulocytes) with 10^{-7} M insulin for 3 days, followed by T-cell separation and activation by phytohemagglutinin, did not reduce insulin-binding activity of treated T-lymphocytes when compared with control cohorts. Thus, our studies clearly demonstrate that reduction in T-lymphocyte insulin-binding activity is mediated directly through the insulin receptor of the T-lymphocyte rather than being influenced by accessory cell types such as monocytes, whose complement of insulin receptors does not require activation for their detection.

Helderman et al.[25] have recently reported that in vivo alterations of insulin levels ultimately influence the number of insulin receptors on mitogen-activated T-lymphocytes developed in culture. They postulated three possibilities to explain these findings: (1) the presence of nondetectable insulin receptors on peripheral blood T-lymphocytes that are capable of downregulation in vivo, (2) education of T-lymphocytes to produce fewer insulin receptors by peripheral blood monocytes that bore insulin receptors and were downregulated, and (3) alterations in plasma fatty acids that, on incorporation into T-lymphocyte membranes, might influence insulin binding activity. Our in vitro studies make possibilities (1) and (2) less likely. Even if cryptic receptors were present in vivo, downregulated cells are fully capable of restoring their complement of insulin receptors after 24 h of culture in an insulin-depleted media. As noted earlier, monocytes exposed to high concentrations of insulin in vitro do not influence the number of insulin receptors expressed on T-lymphocytes after mitogen activation.

Several studies from this laboratory now clearly implicate local environment plasma membrane fluidity as an influential process on insulin receptor-ligand interactions. Membrane fluidity can be easily altered by plasma membrane incorporation of fatty acids in various states of saturation. This may be accomplished by both in vitro or in vivo manipulations.[26-28] Additionally Kamada and Otsuji[29] have recently demonstrated in vivo a negative correlation between levels of high-density lipoproteins and the erythrocyte membrane fluidity of diabetic subjects when compared with nondiabetic controls. We have recently found that prolonged culture of

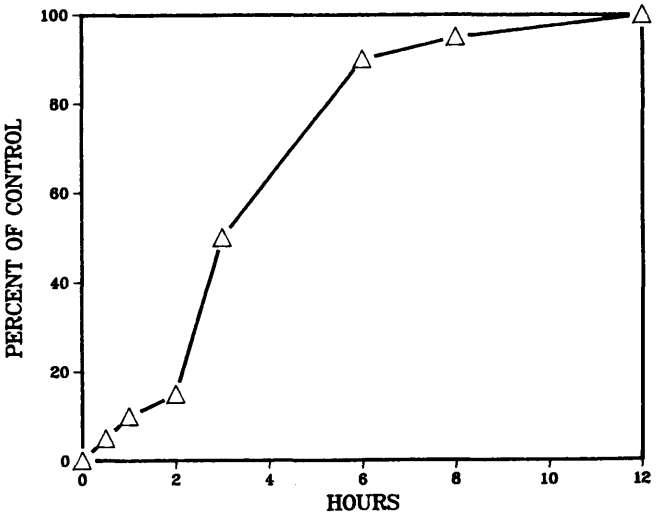

FIGURE 6. Recovery from insulin-induced desensitization. Activated T-lymphocytes from two individuals were cultured with or without insulin 10^{-7} M for 2 h. These cells were subsequently washed to remove cell surface insulin, and then cultured in insulin-depleted media. After varying culture times, aliquots of cells treated with or without insulin were removed and examined for insulin-induced increases in glucose transport when compared with control cells as described in the text. Results are expressed as means of two experiments.

TABLE 2
Effect of insulin on glucose oxidation of activated T-lymphocytes*

	CO_2 generation (pmol/10^6 cells)
Naive cells†	169.0 ± 1.13
Naive cells + 10^{-9} M insulin	192.8 ± 7.82
Desensitized cells‡	170.0 ± 4.16
Desensitized cells + 10^{-9} M insulin	166.0 ± 6.35
Desensitized cells + 10^{-5} M spermine	213.3 ± 0.09

*Matched, activated T-lymphocytes from three individuals were examined for glucose oxidation as described in the text.
†Naive cells are activated T-lymphocytes that have not been previously treated with insulin, but have been exposed to buffer for 2 h at 37°C as described in the text.
‡Desensitized cells are activated T-lymphocytes that have been treated with 10^{-7} M insulin for 2 h at 37°C. These cells were then washed to remove bound insulin as described in the text.

T-lymphocytes with linoleic acid increases these cells' bulk plasma membrane fluidity. These cells' detectable insulin receptors increased 10-fold and correspondingly displayed diminished affinity for ligand (unpublished observations). Hence, it is possible that alterations in T-lymphocyte insulin receptors seen after in vivo insulin manipulations may be due to indirect effects of plasma constituents such as fatty acids. If this postulate is correct, the altered in vitro insulin-binding activity of activated T-lymphocytes demonstrated in diabetic subjects after in vivo insulin or glucose manipulations may still accurately reflect altered insulin-binding activity on their less readily available cell types (adipocytes, hepatocytes, myocytes) in vivo. We are currently investigating the latter possibility.

These studies demonstrated that insulin, in high concentrations, initially stimulated the intermediary metabolism of activated T-lymphocytes; however, on prolonged exposure to insulin these cells rapidly became unresponsive and then were hyporesponsive to insulin. After 2 h, cell surface insulin receptors were reduced by only 10–15%; however, these cells' basal intermediary metabolism was inhibited by insulin, an effect clearly out of proportion to the number of receptors lost. These findings are similar to earlier observations by Marshall and Olefsky[9] that desensitization of murine adipocytes to insulin's effects can occur by postreceptor-mediated mechanisms. As such, our findings are the first to demonstrate these effects in a primary human tissue. Karlsson et al.[30] have demonstrated a similar state of hyporesponsiveness in 3T3-L1 adipocytes induced by anti-insulin receptor antibodies. However, even these hyporesponsive cells' glucose oxidation could be stimulated by spermine (an agent that acts distally to the insulin receptor site by hydrogen peroxide-mediated mechanisms).[22,23] Our findings that spermine can maximally stimulate insulin-induced, hyporesponsive T-lymphocyte glucose oxidation further suggest that desensitization of these cells may be due to an early postreceptor defect. Recovery from the hyporesponsive state required a 6-h incubation of T-lymphocytes in the absence of insulin. After 6 h, T-lymphocyte complement of insulin receptors was reduced by 50%; however, since these cells were now fully stimulable by insulin, these findings further emphasize the importance of postreceptor- rather than receptor-mediated mechanisms as the principal cause of decreased cellular responsiveness to insulin.

Insulin increases cyclic GMP levels in classic target tissues such as adipocytes and hepatic cells.[31] As increases in cyclic GMP levels increase T-lymphocyte cytotoxicity against cells bearing allogeneic determinants, insulin has been indirectly implicated as an immunoregulatory molecule for activated T-lymphocytes.[2,32] However, in recent studies from this laboratory, we have been unable to demonstrate any effect of insulin on cytosolic cyclic GMP or AMP levels in this cell type before or after acquisition of its insulin receptor. Helderman has recently suggested that insulin may be immunoregulatory in view of its ability to mediate increases in the intermediary metabolism of these cells, thereby increasing their ability to mediate cytotoxicity against target cells bearing allogeneic determinants.[17] Interestingly, both Strom and Helderman noted that insulin's effects were extremely time- and dose-dependent.[2,32] Greater than 8 min exposure of effector cells to physiologic concentrations of insulin reduces and then ablates enhanced cytotoxicity, whereas enhanced cytotoxicity is not demonstrable with pharmacologic amounts of insulin. Similarly, Bar et al.[33] have demonstrated that incubation of monocytes with pharmacologic amounts of insulin for >2 h inhibits these cells' cytotoxicity against antibody-coated erythrocytes. The mechanisms to account for these paradoxical findings have not been determined. However, in view of our studies, rapid desensitization of cells by insulin might be considered as one possible explanation for them. Insulin might be viewed as an immunoregulatory molecule only insofar as its effects on intermediary metabolism may positively or negatively influence effector functions of activated mononuclear cell populations that bear insulin receptors. We are currently investigating these possibilities.

In summary, our findings suggest that the activated human T-lymphocyte is an insulin-sensitive tissue that, like other target tissues, is capable of modulating its physiologic response to insulin by receptor- and postreceptor-mediated mechanisms. As such, this cell type appears to be an appropriate model for the study of insulin action in man.

ACKNOWLEDGMENTS

We thank John Nolan for expert technical assistance. We also thank Drs. J. Harold Helderman and Stephen Marshall for generous and helpful advice.

This study was supported by Veterans Administration research funds, by NIH grant AM-25295 as a pilot project of the Diabetes-Endocrinology Research Center, and by a grant from the American Diabetes Association, Iowa Affiliate, Inc., and The American Cancer Society, IN 122C.

REFERENCES

[1] Krug, U., Krug, F., and Cuatrecasas, P.: Emergence of insulin receptors on human lymphocytes during in vitro transformation. Proc. Natl. Acad. Sci. USA 1972; 69:2604–608.

[2] Helderman, J. H., and Strom, T. B.: Emergence of insulin receptors upon alloimmune T-cells in the rat. J. Clin. Invest. 1977; 59:338–44.

[3] Helderman, J. H., Reynolds, T. C., and Strom, T. B.: The insulin receptor as a universal marker of activated T-lymphocytes. Eur. J. Immunol. 1978; 8:589–95.

[4] Helderman, J. H., and Strom, T. B.: Role of protein and RNA synthesis in the development of insulin binding sites on activated thymus-derived lymphocytes. J. Biol. Chem. 1979; 54:7203–207.

[5] Ercolani, L., Brown, T. J., and Ginsberg, B. H.: Tunicamycin blocks the emergence and maintenance of insulin receptors on mitogen activated human T-lymphocytes. Metab. Clin. Exp. 1983; 33:309–16.

[6] Brown, T. J., Ercolani, L., and Ginsberg, B. H.: Properties and regulation of the T-lymphocyte insulin receptor. J. Receptor Res. 1983; 3:481–94.

[7] Rodbell, M.: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. 1964; 239:375–80.

[8] Livingston, J. N., Purvis, B. J., and Lockwood, D. H.: Insulin dependent regulation of the insulin sensitivity of adipocytes. Nature 1978; 273:394–96.

[9] Marshall, S., and Olefsky, J. M.: Effects of insulin incubation on insulin binding, glucose transport, and insulin degradation by isolated rat adipocytes: evidence for hormone-induced desensitization at the receptor and postreceptor level. J. Clin. Invest. 1980; 66:763–72.

[10] Kahn, C. R.: Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metab. Clin. Exp. 1978; 27:1893–902.

[11] Boyum, A.: Separation of leukocytes from blood and bone marrow. Scand. J. Clin. Lab. Invest. 1968; 21 (Suppl. 97):77–89.

[12] Yam, L. T., Li, C. Y., and Crosby, W. H.: Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol. 1971; 55:283–90.

[13] Lioubin, M. N., Meier, M. D., and Ginsberg, B. H.: A rapid, high-yield method of producing mono-[^{125}I]A_{14}-iodoinsulin. In press. Prep. Biochem. 1984.

[14] DeMeyts, P., and Roth, J.: Cooperativity in ligand binding: a new graphic analysis. Biochem. Biophys. Res. Commun. 1975; 66:1118–26.

[15] Thakur, A. K., and Rodbard, D.: Graphical aids to the interpretation of Scatchard plots and dose-response curves. J. Theor. Biol. 1979; 80:383–403.

[16] Kosmakos, F. C., and Roth, J.: Insulin-induced loss of insulin receptors in IM-9 lymphocytes. J. Biol. Chem. 1980; 255:9860–69.

[17] Helderman, J. H.: Role of insulin in the intermediary metabolism of the activated thymic-derived lymphocyte. J. Clin. Invest. 1981; 67:1636–42.

[18] Gavin, J. R., Roth, J., Neville, D. M., DeMeyts, P., and Buell, D. N.: Insulin-dependent regulation of insulin receptor concentrations: a direct demonstration in cell culture. Proc. Natl. Acad. Sci. USA 1974; 71:84–88.

[19] Blackard, W. G., Guzelian, P. S., and Small, M. E.: Downregulation of insulin receptors in primary cultures of adult rat hepatocytes in monolayer. Endocrinology 1978; 103:548–52.

[20] Green, A., and Olefsky, J. M.: Evidence for insulin-induced internalization and degradation of insulin receptors in rat adipocytes. Proc. Natl. Acad. Sci. USA 1981; 79:427–31.

[21] Czech, M. P., Lawrence, J. C., and Lynn, W. S.: Hexose transport in isolated brown fat cells, a model system for investigating insulin action on membrane transport. J. Biol. Chem. 1974; 249:5421–27.

[22] Livingston, J. N., Gurny, P. A., and Lockwood, D. H.: Insulin-like effects of polyamines in fat cells. J. Biol. Chem. 1977; 252:560–62.

[23] Quostel, M.: Cell Biology and Immunology of Leukocyte Function. New York, Academic Press, 1979:1–60.

[24] Marshall, S., and Olefsky, J. M.: Characterization of insulin-induced receptor loss and evidence for internalization of the insulin receptor. Diabetes 1981; 50:746–53.

[25] Helderman, J. H., Pietri, A. O., and Raskin, P.: In vitro control of T-lymphocyte insulin receptors by in vivo modulation of insulin. Diabetes 1983; 32:712–17.

[26] Ginsberg, B. H., Brown, T. J., Simon, I., and Spector, A. A.: Effect of membrane lipid environment on the properties of insulin receptors. Diabetes 1981; 30:773–80.

[27] Gould, R., Spector, A. A., and Ginsberg, B. H.: Effect of lipid composition on the properties of the reconstituted insulin receptor. J. Biol. Chem. 1982; 257:477–84.

[28] Ginsberg, B. H., Jabour, J., and Spector, A. A.: Dietary manipulations produce alterations in the lipid composition of Ehrlich ascites cells and their insulin receptor. Biochim. Biophys. Acta 1982; 690:157–64.

[29] Kamada, T., and Otsuji, S.: Lower levels of erythrocyte membrane fluidity in diabetic patients: a spin label study. Diabetes 1983; 32:585–91.

[30] Karlsson, F. A., Van Obberghen, E., Grufeld, C., and Kahn, C. R.: Desensitization of the insulin receptor at an early postreceptor step by prolonged exposure to antireceptor antibody. Proc. Natl. Acad. Sci. USA 1979; 76:809–13.

[31] Illiano, G., Tell, P. E. G., Siegel, M. I., and Cuatrecasas, P.: Guanosine 3′, 5′-cyclic monophosphate and the action of insulin and acetylcholine. Proc. Natl. Acad. Sci. USA 1973; 70:2443–47.

[32] Strom, T. B., Bear, R. A., and Carpenter, C. B.: Insulin-induced augmentation of lymphocyte-mediated cytotoxicity. Science 1975; 187:1206–208.

[33] Bar, R. S., Kahn, C. R., and Koren, H. S.: Insulin inhibition of antibody-dependent cytoxicity and insulin receptors in macrophages. Nature 1977; 265:632–35.