Characteristics of Insulin Receptors and Insulin Action in Human Myelogenous Leukemia Cell Line K-562

TOSHIKAZU YAMANOUCHI, TOSHIO TSUSHIMA, YASUO AKANUMA, MASATO KASUGA, HIDEAKI MIZOGUCHI, AND FUMIMARO TAKAKU

SUMMARY

Specific binding sites for insulin have been identified and characterized for the human erythroleukemia cell line K-562. The binding of [^{125}I]-insulin to the cells increased as a function of time, reaching a maximum at 20 min when incubation was performed at 37°C. The binding of [^{125}I]-insulin was dose-dependently inhibited by insulin or proinsulin. Scatchard plot of the binding data was curvilinear, and the number of insulin receptors was approximately 39,000. Insulin at concentrations of 0.05–10.0 ng/ml stimulated CO_2 production and DNA and protein synthesis in K-562 cells in a dose-dependent manner, indicating that the insulin binding sites are functionally important in mediating these biochemical events induced by insulin. Maximal insulin responses were elicited at concentrations of <5 ng/ml, when (at most) 10% of the insulin receptors were occupied. After binding to the cells, [^{125}I]-insulin was degraded in a time- and temperature-dependent manner. As reported for other types of cells, unlabeled insulin also downregulated insulin receptors in K-562 cells. When the cells were incubated with 1×10^{-7} M unlabeled insulin for 24 h, the number of insulin receptors decreased by 50% without a change of affinity. K-562 cells may be useful in studying the role of insulin receptors in cell functions induced by insulin. DIABETES 1985; 34:347–52.

Specific binding sites for insulin have been identified in a number of types of human blood cells.[1–8] Mature red cells have 40–2000 insulin binding sites per cell. However, the physiologic significance of insulin binding sites in red cells remains to be clarified. Earlier studies showed that the number of insulin receptors changes in association with cell differentiation: reticulocytes[4] have more insulin receptors than do mature red cells.[4,6,8–10] In Friend erythroleukemia cells,[11] differentiation induced by dimethylsulfoxide is accompanied by a decrease in the number of receptors. Galbraith et al.[12] also reported that the number of binding sites on erythroblastic leukemia cells (EBL) is 10-fold greater than that on mature erythrocytes. They showed that insulin stimulated the transport of α-aminoisobutyric acid and the incorporation of uridine into RNA in EBL.

K-562 is a human erythroleukemia cell line established by Lozzio,[13] and its differentiation into more mature cell types can be induced by a number of reagents.[14–16] Thus, this cell line provides a useful model for cell differentiation. To our knowledge, there have been no reports made on the characteristics of insulin receptors on K-562; therefore, we have studied the interaction of insulin with this cell line. We have shown that a low concentration of insulin stimulates glucose uptake and DNA and protein synthesis in K-562 cells.

MATERIALS AND METHODS

Hormones and chemicals. Monocomponent porcine insulin was obtained from Eli Lilly and Company (Indianapolis, Indiana). Human insulin, porcine proinsulin, and porcine glucagon were gifts of Eli Lilly and Company. Epidermal growth factor (EGF) (lot 83-111) and multiplication-stimulating activity (MSA) (lot 83-226) were purchased from Collaborative Research Inc. (Lexington, Massachusetts). [^{125}I]-insulin was prepared by the chloramine-T method modified as reported[1] to a specific activity of 100 mCi/mg. [D-1-^{14}C]-glucose (59.8 mCi/mmol), [^{14}C]-thymidine (20 Ci/mmol), and [^{3}H]-leucine (59 Ci/mmol) were purchased from New England Nuclear (Boston, Massachusetts). Crystalline TCA, hyamine hydroxide, and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (Hepes) were purchased from Sigma Chemical Co. (St. Louis, Missouri).

Cells and cell culture. K-562 cells were provided by Dr. S. Sato, the National Cancer Research Institute (Tokyo, Japan).

From the Third Department of Internal Medicine, University of Tokyo, Hongo, Tokyo 113; and the Department of Internal Medicine, Tokyo Women's Medical College, Institute for Growth Science, Kawadacho-10, Ichigaya, Shinjuku, Tokyo 162, Japan.

Address reprint requests to Dr. Toshikazu Yamanouchi, Third Department of Internal Medicine, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

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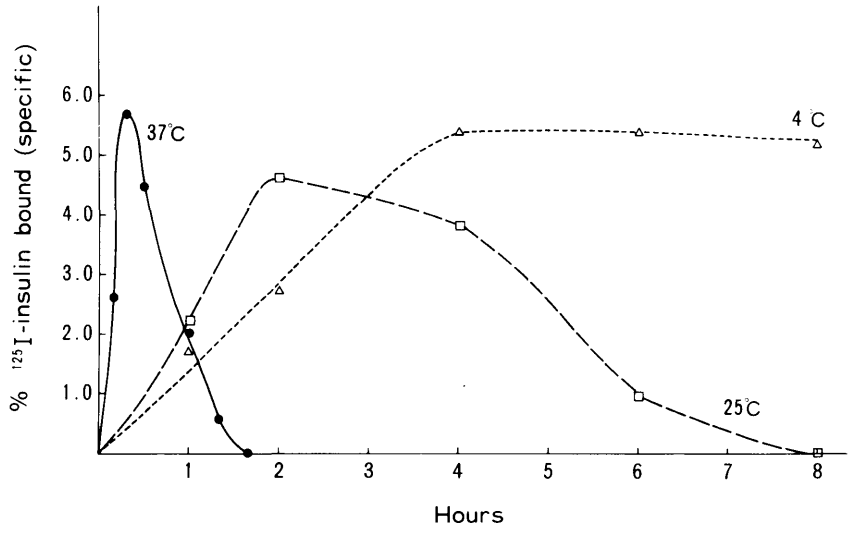

FIGURE 1. Time and temperature dependency of [^{125}I]-insulin binding to K-562 cells: effect of duration of incubation and temperature on the specific binding of [^{125}I]-labeled insulin to the K-562 cell line. Cells (2 × 10^6/ml) were incubated with [^{125}I]-labeled insulin (120 pg/ml) with and without excess unlabeled insulin. At the times indicated, the cells were removed and the specific binding of [^{125}I]-insulin was determined.

Cells were cultured at 37°C in RPMI-1640 medium (Gibco, Grand Island, New York) and supplemented with 10% fetal calf serum (FCS) in a humidified atmosphere of 5% CO_2 in air. All experiments were performed using cells in the late log phase of growth.

Insulin binding study. Cells were allowed to grow to a density of 1.5 × 10^6/ml in 10 ml of medium in 75-cm^2 culture flasks (Falcon). The cells were washed once with the medium and suspended in Hepes binding buffer.[17] An aliquot of the suspension (10^6 cells/tube) was incubated with tracer doses of [^{125}I]-insulin (50,000 cpm, 0.2 ng) in the presence or absence of unlabeled insulin in a total volume of 1 ml of Hepes binding buffer containing 0.5% bovine serum albumin (BSA). The tubes were then centrifuged (3000 rpm for 30 min) and washed four times with ice-cold, Krebs-Ringer-Hepes buffer containing 0.1% BSA, and the pellets counted to determine the specific binding of [^{125}I]-insulin. Cell viability, as assessed by trypan blue dye exclusion, was always >90% at the end of incubation. Nonspecific binding was defined as the radioactivity obtained in the presence of 1 μg/ml unlabeled insulin.

DNA and protein synthesis. Cells from the stock culture were washed twice with serum-free RPMI-1640 medium and suspended in the serum-free medium at a density of 10^5/ml. After 24 h, insulin (0–10 ng/ml) was added and the dishes were incubated for another 16 h. The cells were then pulsed with [^{3}H]-thymidine (1 μCi/ml) for 2 h. After the cells were washed twice with ice-cold, phosphate-buffered saline (pH 7.4, without Ca^{2+} or Mg^{2+}), the [^{3}H]-thymidine incorporated into TCA-insoluble materials was measured as previously described.[18] [^{3}H]-leucine (1 μCi/ml) incorporation was measured in the same manner except that K-562 cells were exposed to insulin for 6 h. The values were calculated as dpm/mg protein and expressed as a percentage above control (unstimulated) values.

CO_2 production study. The production of CO_2 from glucose substrates by K-562 cells was estimated by the modified method of MacLennan and Golstein.[19] K-562 cells were washed once with Krebs-Ringer bicarbonate buffer containing BSA (1 mg/ml) and 1 mM glucose, pH 7.2, and resuspended in the same buffer. One milliliter aliquots of the suspension containing 2 × 10^6 cells/ml were added to flasks

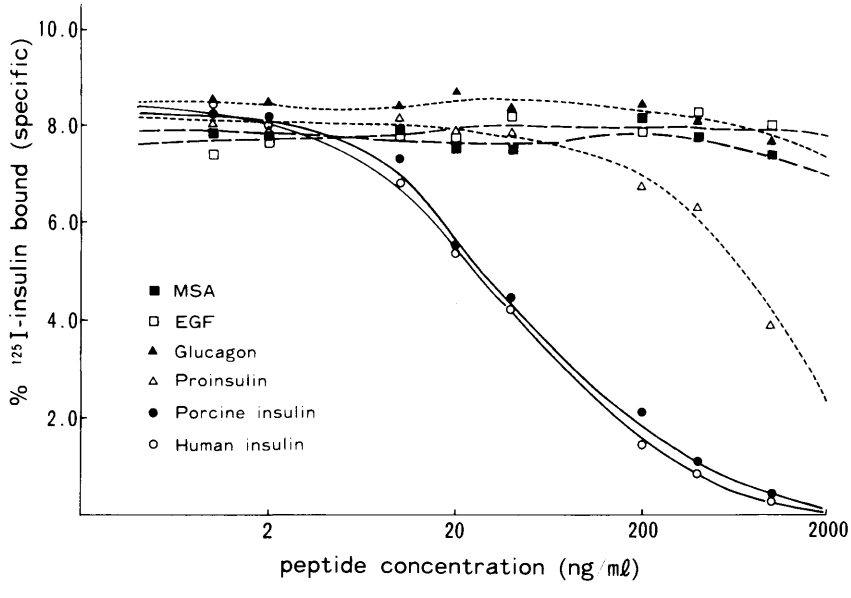

FIGURE 2. Percentage and specificity of [^{125}I]-insulin binding to K-562 cells. Cells (2 × 10^6/ml) were incubated with [^{125}I]-labeled insulin (2 × 10^{-10} M) for 16 h at 4°C in a binding buffer, pH 7.6, in the presence of increasing concentrations of unlabeled polypeptide hormones: multiplication-stimulating activity (MSA) (■), epidermal growth factor (EGF) (□), porcine glucagon (▲), porcine proinsulin (△), porcine insulin (●), and human insulin (○). The tubes were then centrifuged and the radioactivity of the cell pellet counted. The nonspecific binding obtained in the presence of 1000 ng/ml insulin has been subtracted from all values.

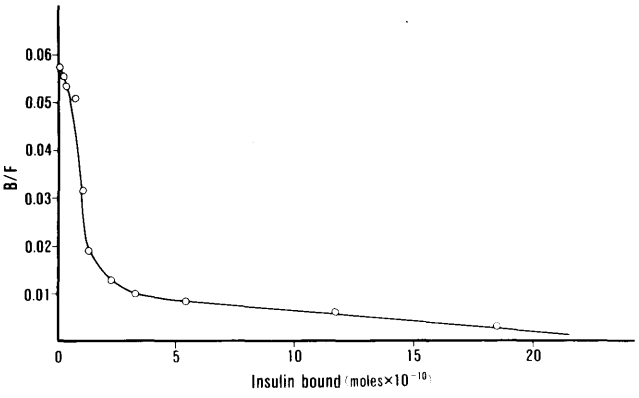

FIGURE 3. Scatchard plots of insulin binding to K-562 cells. Data were derived from Figure 1.

containing 0.1 μCi/ml (final) 1-^{14}C-glucose and various concentrations of insulin standard. The mixture was then incubated at 37°C in 20-ml scintillation flasks for 1 h. The flasks were stoppered by caps fitted with cups (Kontes Glass Company, Vineland, New Jersey) that contained filter paper wicks impregnated with 200 μl hyamine hydroxide. At the end of the incubation period, 0.5 ml of 8 N H_2SO_4 was injected through the stopper into the incubation medium. The flasks were then incubated for another hour at 37°C in a shaking water bath to permit the CO_2 produced in the reaction to be trapped by the hyamine. The cups were removed and assayed for radioactivity.

Insulin degradation. The cell-free supernatant obtained from the insulin binding study was analyzed for degradation of [^{125}I]-insulin. The integrity of [^{125}I]-insulin appearing in the medium was assessed by its solubility in TCA and its ability to rebind to K-562 cells.[20] Results were compared with those of appropriate control tubes that were incubated identically but without cells.

Downregulation. Cells were cultured in a RPMI-1640 medium containing 10% FCS for 24 or 48 h with or without 1×10^{-7} M insulin. The cells were then washed with phosphate-buffered saline (PBS), pH 7.0, at 30°C for 30 min to dissociate surface-bound insulin.[21] The procedure was repeated four times; the binding of [^{125}I]-insulin was then determined as described above.

RESULTS

The binding of [^{125}I]-labeled insulin was dependent on time and temperature (Figure 1). Binding at 37°C was rapid and reached a maximal level within 20 min, followed thereafter by a rapid decline. At 25°C, the rate of association was slower and maximal binding was obtained at 2 h. At 4°C, a steady state was achieved at 4 h. Unless otherwise noted, the binding study was performed at 4°C to minimize the degradation of [^{125}I]-insulin. The specificity of [^{125}I]-insulin binding is shown in Figure 2. Porcine and human insulin inhibited, with identical potencies, the binding of [^{125}I]-insulin, and porcine proinsulin was approximately 1/50 as potent as pork insulin. Multiplication-stimulating activity (MSA) inhibited binding only at very high concentrations. Epidermal growth factor (EGF) and porcine glucagon were without effect (Figure 2).

The Scatchard plot of the data was curvilinear, based on the assumption that the binding was in a steady state (Figure 3). The mean insulin receptor concentration was estimated to be around 39,000/cell.

The insulin degradation process was also time and temperature dependent, as shown in Figure 4. At 4°C, most of the labeled hormone in the medium remained intact for up

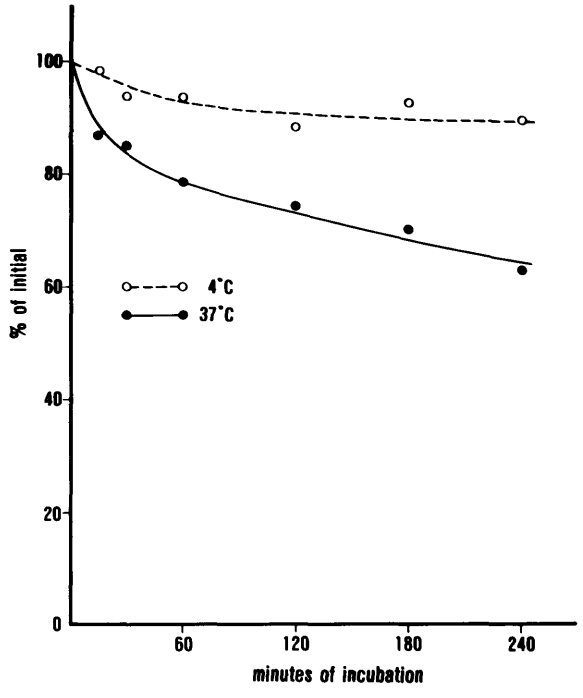

FIGURE 4. Integrity of [^{125}I]-insulin in medium (% of initial): effect of temperature on the degradation of [^{125}I]-insulin during incubation with K-562 cells. [^{125}I]-insulin degradation was assessed by its solubility in cold TCA. K-562 cell concentration was 4 × 10^6/ml of the incubation medium.

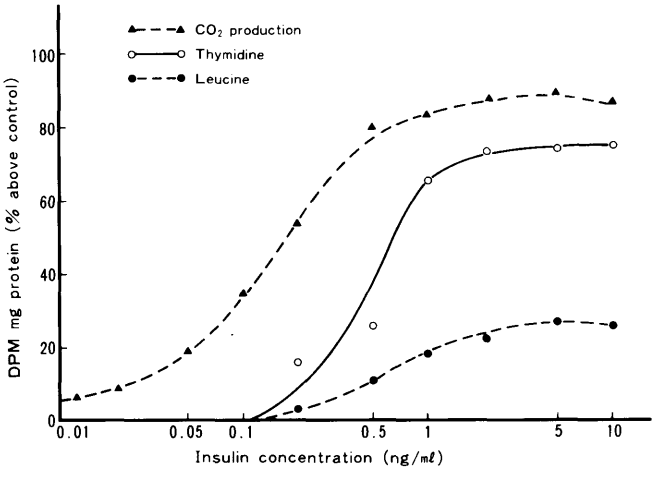

FIGURE 5. The effect of insulin on glucose oxidation and thymidine and leucine incorporation. Insulin at indicated concentrations was added to cells previously maintained in serum-free medium for 24 h. [^{14}C]-thymidine or [^{3}H]-leucine was added to the cells at 16 and 6 h after the insulin, respectively, and pulsed for 2 h. After harvesting the cells by centrifugation, the [^{3}H]-leucine and [^{14}C]-thymidine incorporated into TCA-insoluble material were determined. CO_2 production was assessed as described in MATERIALS AND METHODS, and the specific radioactivity of [D-1-^{14}C]-glucose was determined. Values are the mean of three or five experiments, each done in triplicate.

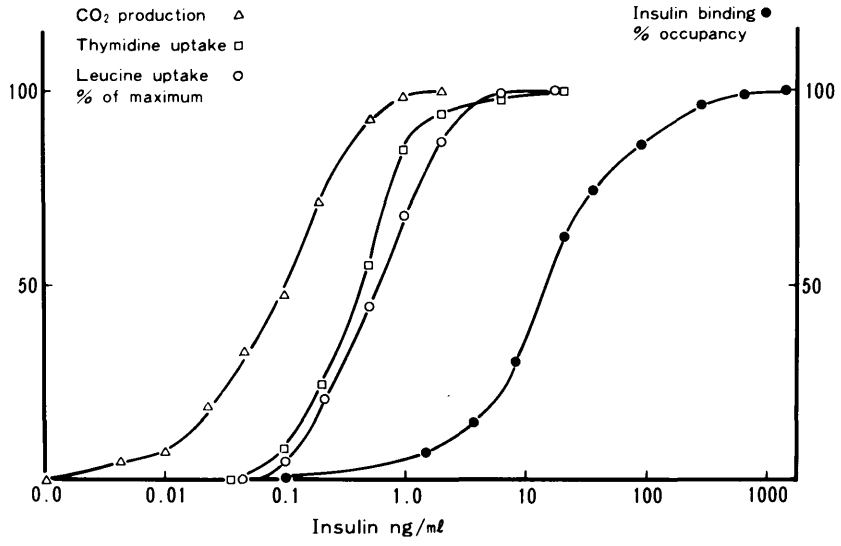

FIGURE 6. Dose-response curves for the effect of insulin on CO_2 production and thymidine and leucine uptake, and for the binding of insulin to K-562 cells. Each effect and insulin binding are expressed as percent of maximal effect and percentage of receptor occupancy, respectively.

to 4 h, as judged by its TCA solubility. On the other hand, 40% of the labeled hormone in the medium was TCA soluble after 4 h of incubation at 37°C.

The effects of insulin on the metabolism of K-562 cells were examined (Figure 5). It can be seen that an amount of insulin as low as 0.2 ng/ml stimulated thymidine incorporation into DNA. Maximal stimulation was observed at 5 ng/ml of insulin. The incorporation of leucine into protein was stimulated by insulin in a similar manner. Glucose oxidation was much more sensitive to insulin. A final concentration of insulin as low as 0.01 ng/ml elicited a significant biologic effect, and maximal effect was obtained with a 0.5 ng/ml dose of insulin. Insulin exerted an enhancing effect on CO_2 production, at rates from 0.61 ± 0.08 to 1.12 ± 0.16 nmol/10^6 cells · h ($N = 5$, $P < 0.01$). The dose-response curve for insulin binding was compared with that for insulin action, glucose oxidation, thymidine uptake, and leucine uptake (Figure 6). It can be seen that occupation of only 5% of the insulin receptors is sufficient to produce maximal glucose oxidation, and approximately 10% occupancy was required for maximal responses in thymidine and leucine uptake.

As reported for insulin receptors on other blood cells (IM-9 lymphocytes,[21] rabbit reticulocytes,[4] and Friend erythroleukemia cells[4]), insulin downregulated its own receptors in K-562 cells. The number of high-affinity receptors decreased to 50% and 20% of the initial number after 24 and 48 h, respectively, when the cells were incubated in the presence of 1×10^{-7} M of insulin (Figure 7).

DISCUSSION

Insulin receptors have been identified in a number of peripheral blood cells, such as mature red cells,[4,6,8-10] reticulocytes,[4] and monocytes.[1] Furthermore, it has been reported that several lines of leukemic cells possess insulin receptors.[11,12,22] The data presented here demonstrate that K-562 cells have insulin receptors that may be functionally important. K-562 cells can be induced to differentiate, mainly to early erythroblasts that can synthesize embryonic hemoglo-

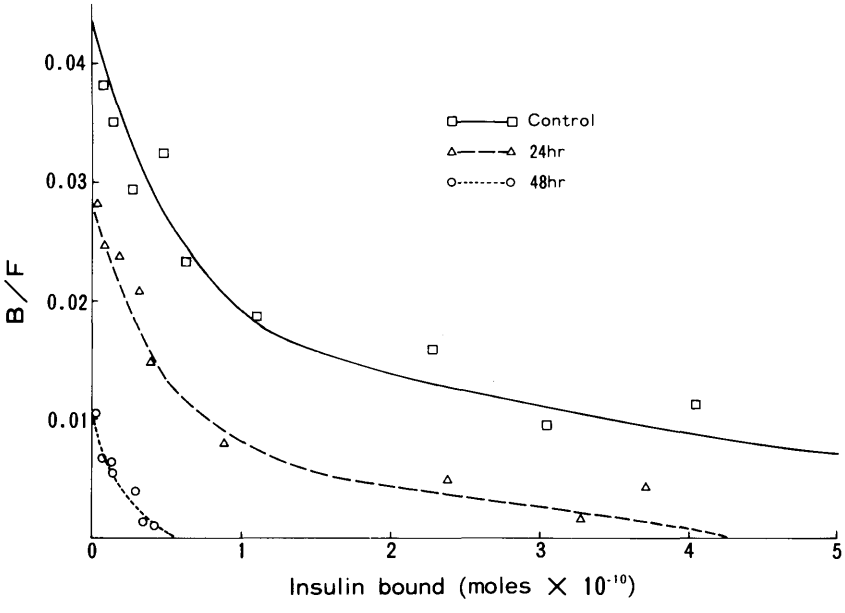

FIGURE 7. Change in the number of insulin receptors under different culture conditions. K-562 cells were cultured for 1 h (□), 24 h (△), or 48 h (○) with insulin (1×10^{-7} M). After the cells were washed as described in MATERIALS AND METHODS, the specific binding of [^{125}I]-insulin was determined. Values are the means of triplicate determinations.

bin.[14–16] The number of insulin binding sites on K-562 (39,000/cell) is much greater than that reported for mature red cells or reticulocytes and is similar to that of Friend leukemia cells[11] or erythroleukemia cells.[12] This observation is compatible with a previous report made by Galbraith[12] that immature erythroid cells lose insulin receptors in association with cell differentiation or maturation.

The binding of [^{125}I]-insulin to the receptors was time and temperature dependent. At 37°C, the binding peaked at 20 min and then rapidly declined. In contrast, incubation at 4°C required more than 4 h to reach equilibrium. The maximal binding at 37°C and 20 min was almost identical to that at 4°C and 4 h. We show that [^{125}I]-insulin is degraded as a function of incubation time, as judged by the TCA precipitability of [^{125}I]-labeled materials appearing in the medium. The process was also dependent on temperature, and only a small amount of degradation was detected at 4°C. [^{125}I]-insulin showed almost the same extent of degradation during 20 min of incubation at 37°C as that during incubation for 4 h at 4°C, which may account for the equal amount of [^{125}I]-insulin binding at the two conditions. However, the rapid loss of insulin binding at 37°C cannot be accounted for by the inactivation of [^{125}I]-insulin alone, since only 25% of [^{125}I]-labeled materials in the medium were TCA soluble at 2 h, at which time binding was essentially nil. From these results, degradation or downregulation of insulin receptors, as well as [^{125}I]-insulin degradation, might be responsible for the rapid decrease in insulin binding at 37°C. The binding of [^{125}I]-insulin was dose-dependently inhibited by unlabeled insulin, the half-maximal inhibition being attained at 20 ng/ml. The Scatchard plot derived from the binding data was curvilinear, suggesting heterogeneity or a negative cooperativity of insulin receptors.

Like insulin receptors in other types of cells,[4,21,23–25] insulin downregulated its own receptors in K-562 cells; downregulation does not occur in mature red cells that are unable to synthesize protein.[21] Our results are compatible with the hypothesis proposed by Ginsberg et al. that protein synthesis is required for downregulation.[4] We showed that K-562 cells responded to physiologic concentrations of insulin with an accelerated uptake of glucose, as well as increases of both protein and DNA synthesis. These biologic responses were triggered by a low percentage of receptor occupancy. Only 5%, 10%, and 10% occupancy levels were required to elicit maximal responses for glucose oxidation and protein and DNA synthesis, respectively. It is remarkable that a very low (0.01 ng/ml) concentration of insulin is able to accelerate glucose oxidation.

In preliminary experiments, lactic acid production of this cell line was also stimulated by similar small dose of insulin. The ability of insulin to act as a growth factor has been reported in a number of cell types in culture. Insulin promotes DNA synthesis and mitosis in chick fibroblasts,[26] thymidine uptake in rat hepatoma H35 cells,[27] and growth of F9 embrional carcinoma cells.[28] In addition, insulin (2–1000 ng/ml) initiates DNA replication and accelerates glucose uptake in Swiss 3T3 cells in the presence of prostaglandins.[29] Galbraith et al.[12] reported that insulin stimulates α-aminoisobutyric acid transport and RNA synthesis in EBL cells. Except in H35 rat hepatoma cells, however, supraphysiologic concentrations of insulin are required to reach a maximal response. Since insulin at high concentrations can compete with specific insulin-like growth factor (IGF) receptors for binding, it is possible that the growth-promoting effects of insulin are mediated through its binding to IGF receptors. King et al.[30] have reported that the mitogenic action of insulin on human fibroblasts is a result of interaction with IGF receptors. In the present study, however, DNA synthesis of K-562 cells was stimulated by very low concentrations of insulin. The half-maximal response obtained with 0.5 ng/ml of insulin indicates that the response is quite sensitive, and such sensitivity suggests mediation of the response through the insulin receptor, not through the IGF receptor. In preliminary experiments, we found specific receptors for IGF-I on K-562 cells. The binding of [^{125}I]-IGF-I was not inhibited by insulin except at very high concentrations (>1000 ng/ml), and, thus, it is unlikely that insulin at low concentrations exerts its effect by interacting with IGF-I receptors. These findings strongly suggest that insulin may be important in growth or other functions of K-562 cells. It remains to be determined whether insulin plays a critical role in the growth or maturation of normal erythroid stem cells. In conclusion, we have demonstrated the presence of specific receptors for insulin and the biologic responses elicited by low concentrations of insulin in K-562 cells. K-562 cells provide a useful model for the study of receptor functions, and in addition, the cells can be used as a sensitive bioassay for insulin.

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