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CHARACTERIZATION OF INSULIN RECEPTORS IN HUMAN PROMYELOCYTIC LEUKEMIA CELL HL60

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SUMMARY. Highly specific insulin receptors have been identified on human promyelocytic leukemia cells HL60. Insulin binding increased progressively with time to reach a maximum at 2 h at 22° and was proportional to the number of cells in the incubation mixture. Insulin degradation as assessed by TCA precipitation and reincubation studies was negligible. Scatchard analysis of the binding data was curvilinear and the total number of insulin receptor sites per cell was around 45,000. The average affinity profile gave an "unoccupied site" affinity constant of 3.5×10^8 M^{-1}. The promyelocytic cells HL60, thus, have specific binding sites and binding characteristics similar to more mature human myeloid cells.

INTRODUCTION

Insulin has been largely involved in growth and differentiation of tissues and mammary cells (1-4) and specific receptors for this hormone have been demonstrated on cells from circulating human blood including mature granulocytes and monocytes (5-9). Recently, Collins et al. (10) have developed, from the peripheral blood leukocytes of a patient with acute promyelocytic leukemia, a permanent cell line (HL60) which consists predominantly (90-95 %) of promyelocytes. This cell line was shown to develop the cellular functions of mature granulocytes when incubated in dimethyl-sulfoxide or other polar compounds (11) or retinoic acid (12), whereas it developed the cellular functions, surface markers, morphology and adherent properties of monocytes-macrophages when incubated in the presence of certain phorbol esters (13) or leukocyte conditioned medium (14). Thus this cell line provides an invaluable *in vitro* system for the analysis of the events leading to myeloid cell differentiation.

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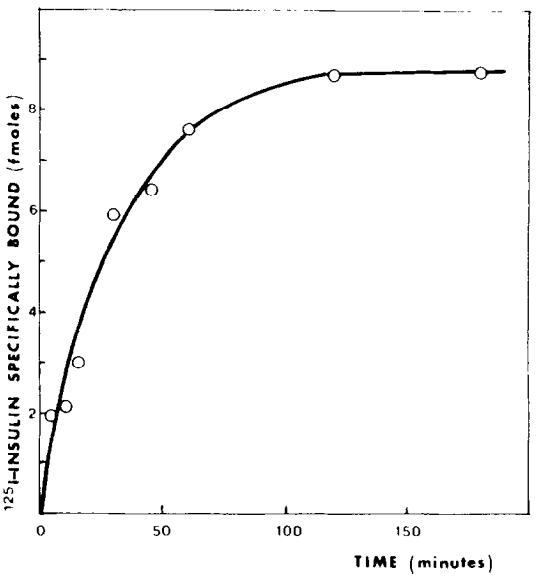

Fig. 1 : Time course of binding of ^{125}I-insulin to HL60 cells. ^{125}I-insulin (2×10^{-10} M) was incubated with HL60 (2×10^{6} cells) in the presence and in the absence of unlabeled insulin (10^{-6} M) at pH 8 in a total volume of 0.2 ml, at 22°. At intervals, aliquots were removed, centrifuged and the radioactivity in the cell pellet counted. The specifically bound ^{125}I-insulin was determined as described in Methods. Each point is the average of three experiments done in duplicate.

In this paper we demonstrate the presence of receptors for ^{125}I-insulin on HL60 cells with specificity analogous to that found on mature human hemopoietic cells as well as other human cell types.

MATERIALS AND METHODS

The human promyelocytic leukemia cells HL60 were kindly provided by Dr R.C. Gallo (National Cancer Institute, Bethesda MD, USA). The cells were grown in continuous liquid suspension culture in RPMI 1640 supplemented with 20 % heat-inactivated fetal calf serum, 2mM L-glutamine, penicillin (100 IU/ml) and streptomycin (100 µg/ml) at an initial concentration of 2×10^{5} cells/ml. Cells were harvested by centrifugation, washed twice and resuspended in incubation buffer containing HEPES 50 mM, Tris-Cl 50 mM, $MgCl_2$ 10 mM, EDTA 2 mM, dextrose 10 mM, $CaCl_2$ 10 mM, NaCl 50 mM, KCl 5 mM and 0.1 % BSA, pH8. Cell viability was assessed by Trypan blue dye exclusion and found to be never less than 90 %.

Binding of ^{125}I-labeled porcine insulin (15) was studied by incubating HL60 cells (1 to 2×10^{6}) with ^{125}I-insulin (2×10^{-10}M) at 22° in 0.2 ml final volume in plastic microcentrifuge tubes. The incubation was started by addition of cells and terminated by dilution with 1 ml ice-cold buffer followed by centrifugation (10s at 12,000rpm). The supernatant was discarded by aspiration and the cell pellet was washed rapidly with 1 ml ice-cold buffer and separated again by 10s centrifugation. The radioactivity of the cell pellet was determined in a Kontron-electronics model CG4000 gamma counter. Non specific binding was defined as the amount of binding not inhibited by 10^{-6} M unlabeled insulin and was usually 20-25 % of the total counts bound. Specific binding was defined as the total amount of ^{125}I-insulin bound minus the non specific binding.

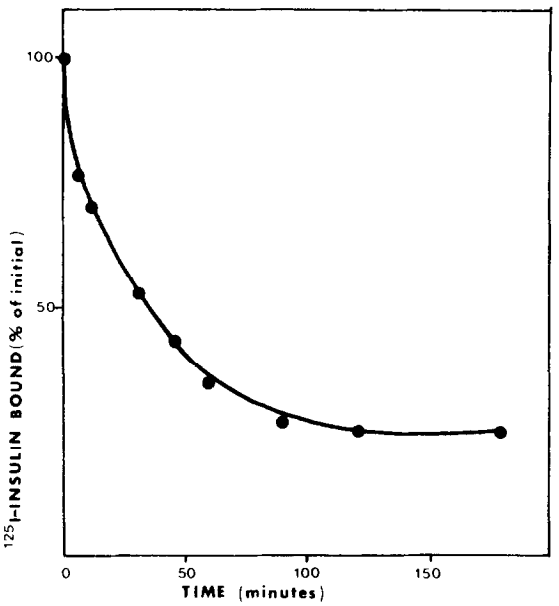

Fig. 2 : Effect of excess insulin on the dissociation rate of ^{125}I-insulin. HL60 cells were incubated as described in Fig. 1. At the end of 2 hours, unlabeled insulin at a final concentration of 10^{-6} M was added. Duplicate aliquots were removed at intervals and bound ^{125}I-insulin was determined as described in Methods.

Pork monocomponent, human insulin, pork proinsulin, pork C-peptide and glucagon were purchased from Novo, Bagovaard, Denmark. RPMI 1640 medium, fetal calf serum, glutamine and antibiotics were from Boehringen, Mannheim. BSA Fraction V was obtained from Miles Laboratories.

RESULTS

The time-course of specific binding of ^{125}I-insulin by HL60 cells at 22° is presented in Fig. 1. The maximum is reached between 2 and 3 h of incubation. Half-maximum binding was observed at 20 min. Binding experiments were performed at pH8.0 because that pH yielded maximum specific binding. Insulin degradation in the medium by HL60 cells was studied by TCA precipitation and rebinding and was found to be negligible (results not shown). The specific binding of ^{125}I-insulin during a 2 h incubation was found to be directly proportional to the concentration of cells until at least 25 x 10^6 cells/ml. Fig. 2 shows that the ^{125}I-insulin bound to HL60 cells after a 2 h incubation at 22° can be totally dissociated by an excess of cold insulin.

When HL60 cells were incubated with 2 x 10^{-10} M ^{125}I-insulin together with increasing concentrations of non-labeled insulin at 22° for 2 h, a

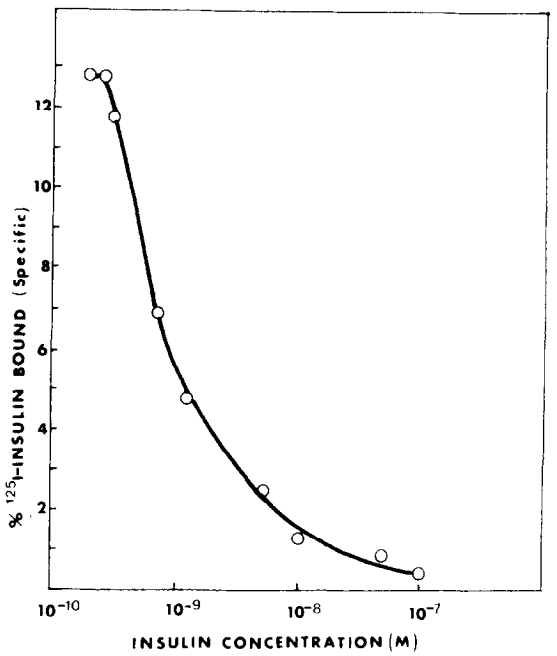

Fig. 3 : Percent of ^{125}I-insulin bound to HL60 cells. Cells were incubated at pH 8, 22°, with ^{125}I-insulin (2 x 10^{-10} M) and various concentrations of unlabeled insulin (0 to 10^{-6} M) in a total volume of 0.2 ml for 2 hours. The tubes were then centrifuged and the radioactivity of the cell pellet counted. The nonspecific binding (in the presence of 10^{-6} M insulin) has been subtracted from all values.

competitive displacement of labeled insulin was observed as shown in Fig. 3. The Scatchard plot of the data based on the assumption that the binding was in a steady state was curvilinear (Fig.4). The mean insulin receptor concentration was estimated to be around 45,000 per cell. From this value and the Scatchard data an average affinity profile was calculated (shown in Fig. 5) according to De Meyts et al. (16). In HL60 cells, the highest or "empty sites" affinity, $\overline{Ke}$, was 0.35 nM^{-1}. With the increasing occupancy of the receptors by insulin, apparent $\overline{K}$ progressively decreases until $\overline{Kf}$ is reached. $\overline{Kf}$ is equal to 0.02 nM^{-1} and is reached when 20 to 30 % of the available receptor sites are occupied.

Fig. 6 shows, as found in insulin binding studies to most systems, that addition of excess cold insulin increases the rate of dissociation of ^{125}I-insulin obtained by a 1:100 dilution only.

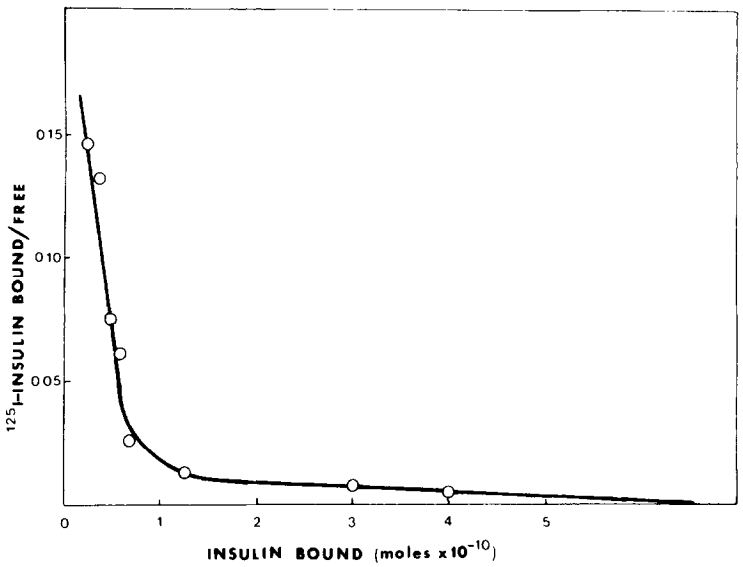

Fig. 4 : Scatchard analysis : data from Fig. 2 were analysed.

To further define the specificity of insulin binding to HL60 cells the displacement of ^{125}I-insulin by non labeled insulins and other peptides were examined. The data shown in Table I indicate that porcine and human

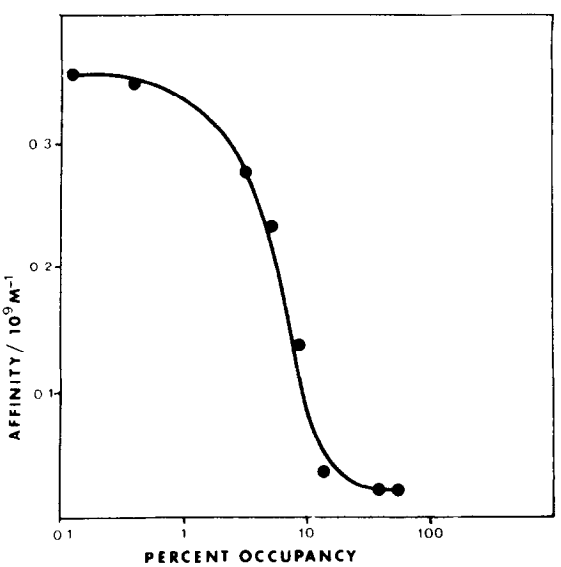

Fig. 5 : Average affinity profile of ^{125}I-labeled insulin binding. The average affinity constant, $\overline{K}$, (B/F/RO-B) is plotted versus the log of the percentage of occupancy of receptor sites ($\overline{Y}$ x 100) calculated by B/RO. B is the amount of insulin bound, RO is the receptor concentration, and $\overline{Y}$ is the fraction of receptor occupancy. The points of the curve were derived from the Scatchard plot (Fig. 4)

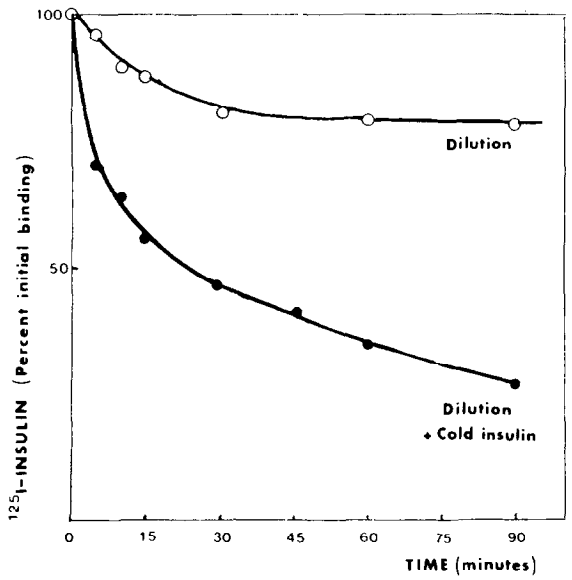

Fig. 6 : Effect of insulin on rate of dissociation of ^{125}I-insulin. HL60 cells were incubated with ^{125}I-insulin (2×10^{-10} M) and allowed to reach steady state by incubation at 22° for 2 hours. Aliquots of 100 µl were then diluted in 10 ml of buffer alone or in 10 ml of buffer containing 10^{-7} M insulin (dilution + cold insulin). The diluted mixtures were left at 22° for different length of time and the residual bound insulin was determined by sedimentation as described. The residual radioactivity bound was expressed as a percent of that bound at steady state.

insulin which have the same biological activity (17) displace porcine ^{125}I-insulin with the same efficacy whereas porcine proinsulin which has only 2-5 % biological activity (18) is 100 times less effective than

TABLE I

Effect of insulin-related and unrelated, biologically active peptides on binding of ^{125}I-insulin by HL60.

Hormone	Concentration	^{125}I-insulin binding
		% of control
None		100
Porcine insulin	1 nM	50
Human insulin	1 nM	50
Porcine proinsulin	1 nM	0
	10 nM	75
Rat insulin	1 nM	0
	100 nM	70
Porcine C-peptide	100 nM	0
Porcine glucagon	100 nM	0

HL60 cells were incubated at pH 8, 22°, for 2 hours with 2×10^{-10} M ^{125}I-insulin with or without various test hormones and the binding examined. Values are the means of triplicate incubations.

insulin in displacing ^{125}I-insulin. Neither porcine C-peptide nor porcine glucagon caused measurable displacement of ^{125}I-insulin.

DISCUSSION

The data presented in this paper indicate that immature human myeloid cells (promyelocytes) contain binding sites specific for insulin. The binding of ^{125}I-insulin occurs at physiologic concentrations and unlabeled procine and human insulins, and to a lesser extent porcine proinsulin were able to displace ^{125}I-insulin, whereas no displacement was observed with unrelated hormones or peptides.

Properties of the HL60 insulin receptor defined in this study, such as pH optimum, high affinity binding constant, and curvilinear Scatchard's plot, were found to resemble those described for other insulin binding cells (6-9, 19, 20). Although our finding (Fig. 6) of enhanced dissociation of ^{125}I-insulin from HL60 cells in the presence of unlabeled insulin could support the possibility of negative cooperativity of the insulin receptor (21, 22), this interpretation has recently been questioned (23) and, on the other hand, Herzberg et al. (24) have demonstrated the presence of two populations of insulin receptors on human erythrocytes.

Although insulin receptors have not been demonstrated in more immature myeloid cells, an effect of insulin, at physiological level, on the proliferation of K562 blast cells has been reported (25) suggesting their presence. On the other hand, the mean insulin receptor concentration found in HL60 cells (45,000) is close to that published for mature human monocytes (15,000 to 50,000) (26,27) whereas a concentration of only 1,000 insulin receptors per human mature granulocyte has been determined by Fussganger et al. (6).

Thus, the disappearance of insulin receptors in the membrane of granulocytic cells seems to be one of the events occurring during differentiation and maturation of these cells in the bone marrow. Such a decrease in the number of insulin receptors during differentiation has been reported for rabbit red cells (28). On the contrary,

the differentiation and maturation of monocytes might not be accompanied by such a decrease. Work is in progress in our laboratory to elucidate these points.

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