

The Absence of Cryptic Insulin Receptors on Resting Lymphocytes

YEHOShUA GOZES, JOANNE CARUSO, AND TERRY B. STROM

SUMMARY

Previous experiments have demonstrated that insulin receptors emerge upon stimulated lymphocytes while resting lymphocytes lack insulin receptors. The appearance of insulin receptors is totally dependent on RNA and protein synthesis. The results suggest that insulin receptors are synthesized *de novo* or that new protein is synthesized that is responsible for activation of the receptor. In this study, we investigated the possibility that cryptic membrane receptors are present before lymphocyte activation. As a precedent, hypertonic salt solutions or enzymatic digestion have been reported to uncover cryptic insulin receptors in liver and fat cell membranes. Similar treatment of lymphocytes failed to reveal cryptic, stereospecific receptor sites, although nonspecific insulin binding did increase. *DIABETES* 30:314-316, April 1981.

A receptor for insulin on the membrane and many tissues, such as fat, liver, cardiac, and skeletal muscle, has been well characterized.¹⁻¹⁰ In these tissues, the hormone supports intermediary metabolism. Insulin receptors are also present in tissues whose metabolism may not be altered by insulin, including fibroblasts,^{11,12} monocytes,¹³ erythrocytes,¹⁴ neural cells,¹⁵ and leukemic lymphoblasts.^{16,17} Unstimulated, freshly prepared, thymus-derived (T) and bursal-equivalent (B) lymphocytes do not have a functional insulin receptor.¹⁸ In contrast to other tissues, insulin receptors on lymphocytes become detectable only after activation by antigen¹⁸⁻²⁰ or mitogen.^{16,19-22} Thus, the lymphocyte provides a useful model system for studying the emergence of hormone receptors.

From the Department of Medicine, Renal Division, Laboratory of Immunogenetics and Transplantation, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

Address reprint requests to Terry B. Strom, Department of Medicine, Renal Division, Laboratory of Immunogenetics and Transplantation, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

Received for publication 29 September 1980.

There are at least two nonmutually exclusive hypotheses which may explain the mechanism by which insulin receptors appear upon activated lymphocytes. First, inactive cryptic insulin receptors may be present in the lymphocyte membrane. Activation may produce only a change in membrane configuration, which permits hormone binding. Alternatively, lymphocyte activation may be linked to *de novo* synthetic events culminating in the appearance of functional receptor units. Recently, evidence has been reported which demonstrates that appearance of T-lymphocyte insulin receptors following mitogenic stimulation requires DNA-dependent RNA synthesis and protein synthesis, although *de novo* DNA synthesis and blast transformation are not necessary.^{21,22}

Nevertheless, cryptic receptors have been demonstrated in membranes harvested from liver or fat cells. Treatment of these tissues with phospholipase C or A, NaCl, LiCl, or CaCl₂ unmasked cryptic binding sites for insulin.^{5-7,23,24} It is possible that cryptic lymphocytic insulin receptors are also "blocked" by phospholipids, despite the fact that Krug and co-workers¹⁶ found that phospholipase C did not reveal specific insulin binding sites on the unstimulated lymphocytes.

This study was undertaken to directly investigate the possibility that cryptic plasma membrane receptors are present before or after lymphocyte activation and that hypertonic salt solutions or enzymatic digestion can uncover these sites and permit ligand binding.

EXPERIMENTAL PROCEDURES

MATERIALS

Hanks' balanced salt solution, RPMI-1640 medium, and fetal calf serum (FCS) were purchased from Microbiological Associates. ¹²⁵I-insulin (90% monoiodinated) 100 Ci/ μ g was purchased from New England Nuclear Corp. Unlabeled, single-peak porcine insulin was a kind gift of Dr. Ronald Chance of the Eli Lilly Co. Bovine serum albumin (BSA) was purchased from Miles Laboratories, Inc. Concanavalin A (Con A) was purchased from Sigma Chemical Co. Phospholipase A and C were purchased from Calbiochem.

METHODS

Lewis × Brown Norway F₁ rat livers or thymi harvested from 3-mo-old rats were teased through 60-gauge steel mesh into Hanks' balanced solution. The resultant preparation was sedimented at 450 × g for 10 min and resuspended in fresh media. Before enzymatic treatment, the cells were suspended in RPMI-1640 medium while cells prepared for hypertonic salt treatment or insulin binding studies were suspended in Hanks' balanced salt solution enriched with 0.1% bovine serum albumin (pH 7.4).

Lymphocyte activation. Thymocytes (10⁷/ml) were cultured in vitro for 72 h in RPMI-1640 supplemented with 10% heat-inactivated FCS and 20 μg/ml Con A.

Salt treatment. Liver cells (5 × 10⁶/ml) or thymocytes (15 × 10⁶/ml) were incubated 30 min at room temperature with either 5–30 mM CaCl₂ or 0.5–2.0 M NaCl.

Membrane preparation. Liver or thymic cell membranes were prepared as previously described by Cuatrecasas.⁶

Insulin binding assay. A modification of the insulin binding assay described by Gammeltoft and Gliemann^{25,26} has been described extensively.^{18–22} The modification permits sedimentation of cells through phthalate oils. There is only 0.04% contamination of pellet by free ligand with recovery of >90% of the cells. In brief, 5 × 10⁶/ml liver cells or 15 × 10⁶/ml thymocytes suspended in Hanks' + 0.1% BSA are incubated with ¹²⁵I-labeled insulin. The assay involves a competitive binding reaction between ¹²⁵I-insulin and unlabeled monocomponent porcine insulin. By such competition, both total and nonspecific isotopic binding may be measured and specific (receptor site) binding can be calculated.

RESULTS

Thymocytes harvested from postweaning rats lack insulin receptors. In contrast, Con A-stimulated thymocytes develop a high-affinity, low-capacity, stereospecific receptor for insulin (binding curve not shown) with properties identical to those previously reported from this laboratory in studies of other activated lymphocytes.^{19,20} After interaction of a saturating concentration of insulin (8.2 μg/ml)^{17–19} with activated thymocytes, total ¹²⁵I-insulin and nonspecific ¹²⁵I-insulin binding were, respectively, 1256 ± 14 and 784 ± 2. Aliquots of these mitogen-stimulated cells were then treated

TABLE 1
Influence of salt treatment on ¹²⁵I-insulin binding to thymocytes

Exp. #	Total binding	Nonspecific binding	Treatment
1	943 ± 465	529 ± 99	—
	2,536 ± 471	2,094 ± 792	NaCl (2 M)
	6,438 ± 1,635	4,473 ± 667	CaCl ₂ (10 mM)
2	422 ± 43	271 ± 16	—
	706 ± 409	349 ± 40	CaCl ₂ (5 mM)
	772 ± 116	965 ± 69	CaCl ₂ (10 mM)
	1,884 ± 139	1,700 ± 73	CaCl ₂ (20 mM)
3	137 ± 54	157 ± 28	—
	2,147 ± 1,323	3,003 ± 801	NaCl (2 M)
	1,006 ± 200	1,035 ± 319	CaCl ₂ (10 mM)
4	453 ± 61	405 ± 51	—
	3,311 ± 378	3,045 ± 131	CaCl ₂ (20 mM)
	4,135 ± 345	3,948 ± 53	CaCl ₂ (30 mM)
5	922 ± 68	731 ± 53	—
	3,285 ± 672	3,423 ± 682	NaCl (2 M)
	6,532 ± 327	5,713 ± 535	CaCl ₂ (10 mM)

TABLE 2
Influence of salt treatment on ¹²⁵I-insulin binding to liver cells

Exp. #	Total binding	Nonspecific binding	Treatment
1	15,039 ± 1,233	6,239 ± 1,322	—
	25,680 ± 2,416	19,808 ± 6,514	NaCl (2 M)
2	6,714 ± 748	3,134 ± 82	—
	14,043 ± 238	10,688 ± 93	NaCl (2 M)

with 2 M NaCl solution in an attempt to reveal cryptic receptors. Salt treatment resulted in increased total binding. However, this increase (1256 ± 14 to 4502 ± 752) was due to nonspecific binding that increased from 784 ± 2 to 4112 ± 1459 following NaCl salt exposure. Hence, specific receptor site binding was essentially unchanged.

In addition, we tested the possibility that the insulin receptors present following stimulation are actually cryptic receptors present in an inactive form before stimulation. Resting thymocytes were subjected to several treatments. Five representative experiments are shown in Table 1. NaCl and CaCl₂ treatment of resting thymocytes caused an increase in total and nonspecific ¹²⁵I-insulin binding but specific insulin binding was unchanged. Liver cells were also treated with NaCl or CaCl₂. As shown in Table 2, salt treatment of liver caused an increase in nonspecific ¹²⁵I-insulin but not specific insulin binding. The effect of hypertonic salt treatment upon the insulin binding properties of thymocytes and liver cells was reversed by sedimenting the cells at 450 × g for 10 min and resuspending the cells in the Hanks' media supplemented with 0.1% BSA (Table 3). Treatment of thymocytes with phospholipase A or C did not alter binding (Table 4). Cuatrecasas et al.⁶ previously reported that hypertonic salt solutions or phospholipase treatment of liver cell membranes expose specific insulin binding sites. Disappointingly, we found salt pretreatment of thymic membranes resulted only in increased nonspecific site binding (Table 5).

DISCUSSION

Krug, Krug, and Cuatrecasas¹⁶ first demonstrated that mitogen-activated lymphocytes express insulin receptors. Helderman and Strom have demonstrated that stimulated T-cells reveal a classic insulin receptor after antigen^{18,19} or mitogen^{19–22} stimulation. The appearance of the insulin receptor does not require de novo DNA synthesis but is totally dependent on mRNA and protein synthesis.²¹ These results suggest that the insulin receptor is synthesized de

TABLE 3
Effect of washing cells after salt treatment on ¹²⁵I-insulin binding

	Total binding	Nonspecific binding	Treatment
Thymus cells	1,214 ± 84	820 ± 41	—
	2,225 ± 159	1,946 ± 41	CaCl ₂ (20 mM)
	1,291 ± 451	827 ± 209	CaCl ₂ (20 mM) washed
Liver cells	11,343 ± 589	5,292 ± 365	—
	24,692 ± 1,541	23,665 ± 1,814	NaCl (2 M)
	10,797 ± 397	7,963 ± 921	NaCl (2 M) washed

TABLE 4
Influence of phospholipase A and C treatment on ¹²⁵I-insulin binding of thymus cells

Exp. #	Total binding	Nonspecific binding	Treatment
1	609 ± 124	402 ± 17	
	527 ± 44	433 ± 137	45' 37°C PLA-50 μg/ml*
	489 ± 24	454 ± 96	45' 37°C PLA-5 μg/ml
2	1,197 ± 24	597 ± 64	
	843 ± 94	878 ± 107	5' 37°C PLC-0.5 μg/ml†
	893 ± 273	545 ± 115	5' 37°C PLC-1 μg/ml
3	376 ± 19	294 ± 74	
	395 ± 138	295 ± 26	10' 37°C PLC-0.05 μg/ml
	470 ± 107	386 ± 40	10' 37°C PLC-1 μg/ml
	539 ± 192	306 ± 59	30' 37°C PLC-0.05 μg/ml
	438 ± 121	332 ± 53	30' 37°C PLC-1 μg/ml

* PLA = Phospholipase A.
† PLC = Phospholipase C.

novo or a new protein is synthesized which is responsible for the insertion of the receptors into the membrane. In this report, we attempted to reveal cryptic insulin receptors upon nonactivated thymocytes. To this end, we utilized means used previously by others to detect cryptic membrane insulin receptors in other tissues. While NaCl and CaCl₂ caused increased total insulin binding in liver cells and thymocytes, this increase was solely due to an increase in nonspecific binding. These results stand in marked contrast to the finding of Cuatrecasas and co-workers,^{7,22,24} who observed an increase in stereospecific insulin binding sites after treatment of liver cell membranes with hypertonic salt solutions. This apparent discrepancy cannot easily be attributed to differences in assay techniques, as only the method of eliminating unbound insulin was variable. The rise in nonspecific binding of ¹²⁵I-insulin after exposure to hypertonic salt solutions may be due to perturbations in the configuration of molecules in the membrane which expose new binding sites. For example, high cation concentration shields charge-reduced repulsion, and electrodynamic forces aggregate intramembranous particles on the membrane.^{27,28} Treatment of thymocytes with phospholipase A or C did not alter ¹²⁵I-insulin binding.

In summary, we find no evidence that resting lymphocytes bear cryptic receptors present in the membrane before activation. It is likely that insulin receptors apparent upon activated T-cells result from de novo synthesis of the receptor protein.

ACKNOWLEDGMENTS

Dr. Strom is the recipient of an NIH Career Development Award. This study was supported by NIH grant AM21094-04.

TABLE 5
Influence of salt treatment on ¹²⁵I-insulin binding to thymic membranes

Total binding	Nonspecific binding	Treatment
440 ± 46	424 ± 45	—
1,312 ± 244	1,329 ± 389	20 mM CaCl ₂
1,987 ± 706	1,509 ± 372	2 M NaCl

REFERENCES

- Cuatrecasas, P.: Interaction of insulin with the cell membrane: the primary action of insulin. *Proc. Natl. Acad. Sci. USA* 63:450-57, 1969.
- Freychet, P., Roth, J., and Neville, D. M.: Insulin receptors in the liver: specific binding of [¹²⁵I]insulin to the plasma membrane and its relation to insulin bioactivity. *Proc. Natl. Acad. Sci. USA* 68:1833-37, 1971.
- Freychet, P., Roth, J., and Neville, D. M., Jr.: Moniodoinsulin: demonstration of its biological activity of binding to fat cells and liver membranes. *Biochem. Biophys. Res. Commun.* 43:400-408, 1971.
- House, P. D. R., and Weidermann, M. J.: Characterization of [¹²⁵I] insulin binding plasma membrane fraction from rat liver. *Biochem. Biophys. Res. Commun.* 41:541-48, 1971.
- Cuatrecasas, P.: Perturbation of the insulin receptor of isolated fat cells with proteolytic enzymes: direct measurement of insulin-receptor interactions. *J. Biol. Chem.* 240:6522-31, 1971.
- Cuatrecasas, P.: Unmasking of insulin receptors in fat cells and fat cell membranes: perturbation of membrane lipids. *J. Biol. Chem.* 246:6532-42, 1971.
- Cuatrecasas, P., Desbuquois, B., and Krug, F.: Insulin-receptor interactions in liver cell membranes. *Biochem. Biophys. Res. Commun.* 44:333-39, 1971.
- Cuatrecasas, P.: Affinity chromatography and purification of the insulin receptor of liver cell membranes. *Proc. Natl. Acad. Sci. USA* 69:1277-81, 1972.
- Olefsky, J., Bacon, V. C., and Baur, S.: Insulin receptors of skeletal muscle: specific insulin binding sites and demonstration of decreased numbers of sites in obese rats. *Metabolism* 25:179-91, 1976.
- Forgue, M. E., and Freychet, P.: Insulin receptors in the heart muscle: demonstration of specific binding sites and impairment of insulin binding in the plasma membrane of the obese hyperglycemic mouse. *Diabetes* 24:715-23, 1975.
- Gavin, J. R. III, Roth, J., Jen, P., and Freychet, P.: Insulin receptors in human circulating cells and fibroblasts. *Proc. Natl. Acad. Sci. USA* 69:747-51, 1972.
- Hollenberg, M. D., and Cuatrecasas, P.: Insulin and epidermal growth factor. Human fibroblast receptors related to deoxyribonucleic acid synthesis and amino acid uptake. *J. Biol. Chem.* 250:3845-53, 1975.
- Schwartz, R. H., Bianco, A. R., Hanwerger, B. S., and Kahn, C. R.: Demonstration that monocytes rather than lymphocytes are the insulin-binding cells in preparation of human peripheral blood mononuclear leukocyte: implications for studies of insulin resistant states in man. *Proc. Natl. Acad. Sci. USA* 72:474-78, 1975.
- Ginsberg, B. H., Kahn, C. R., and Roth, J.: The insulin receptor of the turkey erythrocyte: characterization of the membrane bound receptor. *Biochem. Biophys. Acta* 443:227-42, 1976.
- Havrankova, J., Roth, J., and Brownstein, M.: Insulin receptors are widely distributed in the central nervous system of the rat. *Nature* 272:827-29, 1978.
- Krug, V., Krug, F., and Cuatrecasas, P.: Emergence of insulin receptors on human lymphocytes during in vitro transformation. *Proc. Natl. Acad. Sci. USA* 69:2604-2608, 1972.
- Pollet, R. J., Standaret, M. L., and Haase, B. A.: Insulin binding to the human lymphocyte receptor: evaluation of the negative cooperativity model. *J. Biol. Chem.* 252:5828-43, 1977.
- Helderman, J. H., and Strom, T. B.: Emergence of insulin receptors upon alloimmune T cells in the rat. *J. Clin. Invest.* 59:338-44, 1977.
- Helderman, J. H., and Strom, T. B.: Specific insulin binding site on T and B lymphocytes as a marker of cell activation. *Nature* 274:62-63, 1978.
- Helderman, J. H., Reynolds, T. C., and Strom, T. B.: The insulin receptor as a universal marker of activated lymphocytes. *Eur. J. Immunol.* 8:589-95, 1978.
- 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.* 254:7203-07, 1979.
- Helderman, J. H., and Strom, T. B.: Absence of a role for the cellular exoskeleton in the emergence of the T lymphocyte insulin receptor. *Exp. Cell Res.* 123:119-26, 1979.
- Cuatrecasas, P.: Properties of the insulin receptor of isolated fat cell membranes. *J. Biol. Chem.* 246:7265-74, 1971.
- Cuatrecasas, P.: Properties of the insulin receptor isolated from liver and fat cell membranes. *J. Biol. Chem.* 247:1980-91, 1972.
- Gliemann, J., Østerlind, K., Vinten, J., and Gammeltoft, S.: A procedure for measurement of distribution spaces in isolated fat cells. *Biochem. Biophys. Acta* 286:1-9, 1972.
- Gammeltoft, S., and Gliemann, J. L.: Binding and degradation of ¹²⁵I labeled insulin by isolated fat cells. *Biochem. Biophys. Acta* 320:16-32, 1973.
- Gingell, D.: Electrostatic control of membrane permeability via intramembranous particle aggregation. In *Mammalian Cell Membranes*. Jamieson, J. A., and Robinson, D. M., Eds. London, Butterworth Publishers Inc., 1976, pp. 198-219.
- Elgsaeter, A., and Branton, D.: Intramembrane particle aggregation in erythrocyte ghosts: the effect of protein removal. *J. Cell. Biol.* 63:1018-36, 1974.

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