

Effects of Heparin on Insulin Binding and Biological Activity

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SUMMARY

The effect of heparin, a polyanionic glycosaminoglycan known to alter the function of many proteins, on insulin binding and bioactivity was studied. Cultured human lymphocytes (IM-9) were incubated with varying concentrations of heparin, then extensively washed, and ^{125}I -labeled insulin binding was measured. Heparin at concentrations used clinically for anticoagulation (1–50 U/ml) inhibited binding in a dose-dependent manner; 50% inhibition of binding occurred with 5–10 U/ml. Scatchard analysis indicated that the decrease in binding was due to a decrease in both the affinity and the apparent number of available insulin receptors. The effect occurred within 10 min at 22°C and persisted even after the cells were extensively washed. Inhibition of insulin binding also occurred when cells were preincubated with heparinized plasma or heparinized serum but not when cells were incubated with normal serum or plasma from blood anticoagulated with EDTA. By contrast, other polyanions and polycations, e.g., poly-L-glutamic acid, poly-L-lysine, succinylated poly-L-lysine, and histone, did not inhibit binding.

Heparin also inhibited insulin binding in Epstein-Barr (EB) virus-transformed lymphocytes but had no effect on insulin binding to isolated adipocytes, human erythrocytes, or intact hepatoma cells. When isolated adipocytes were incubated with heparin, there was a dose-dependent inhibition of insulin-stimulated glucose oxidation and, to a lesser extent, of basal glucose oxidation. Although heparin has no effect on insulin binding to intact hepatoma cells, heparin inhibited both insulin binding and insulin-stimulated autophosphorylation in receptors solubilized from these cells. These data indicate that heparin may interact with in-

ulin receptors at concentrations normally used for anticoagulation, altering insulin binding and/or action in various cell types. *Diabetes* 36:163–68, 1987

Heparin is a linear, sulfated glycosaminoglycan with a range of molecular weights between 5000 and 30,000. It consists of repeating units of D-glucosamine and D-glucuronic acid. Due to the polyanionic nature of heparin, it interacts with many proteins and enzymes, some of which it inhibits (e.g., pyruvate kinase, pepsin, and trypsin) and others of which it activates (e.g., lipoprotein lipase and tyrosine hydroxylase) (1). Heparin also has been shown to interact with receptors for low-density lipoprotein (2), glucocorticoids (3), and estrogens (4).

In screening serum and plasma samples for antireceptor antibodies, we observed that, in some cases, normal plasma collected with heparin would produce a false-positive test for antireceptor antibody by inhibiting insulin binding to the test cells (IM-9 lymphocytes). To further explore the possibility that heparin interacts with the insulin receptor, we have analyzed the effect of heparin on insulin binding and bioactivity with various cell types.

MATERIALS AND METHODS

Purified pork insulin was obtained from Elanco (Indianapolis, IN). [γ - ^{32}P]ATP (1.0 mCi/346 nmol) and ^{125}I -labeled insulin were from New England Nuclear (Boston, MA). Normal sera were obtained from healthy volunteers after an overnight fast. Heparin preparations were obtained from Abbott Laboratories (North Chicago, IL; 1000 U/ml; Lot no. 34928AF) and from Sigma (St. Louis, MO; Lot no. 72F-0377) and produced similar effects. Poly-L-glutamic acid, poly-L-lysine, histone, and succinylated poly-L-lysine were all from Sigma. The cultured cell lines used in this study (IM-9, EBVT-1, and Fao) (5–7) were grown in RPMI-1640 (Gibco, Grand Island, NY). The medium was supplemented with 5% fetal bovine serum (Flow Laboratories, Irvine, CA) for Fao cells and 10%

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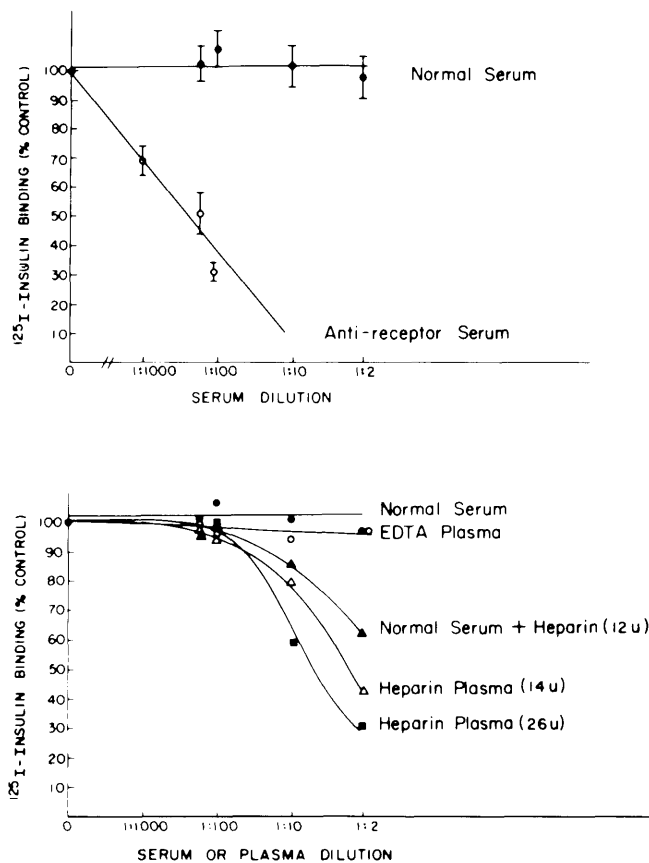


FIG. 1. Top: effect of antireceptor serum on insulin binding to IM-9 lymphocytes. IM-9 lymphocytes (14.6×10^6 cells/ml) were preincubated for 1 h at room temperature (22°C) with increasing dilutions of normal or test serum, extensively washed to remove any substances not tightly bound to cell, and insulin binding was measured. Data are expressed as ^{125}I -insulin-binding percent of untreated control and are means of 3 experiments. Antireceptor antiserum used was from patient B-9, whose serum was anticoagulated with EDTA (16). **Bottom:** effect of serum and plasma on insulin binding to IM-9 lymphocytes. Normal serum plasma collected from blood anticoagulated with EDTA, normal serum to which heparin had been added to give final concentration of 12 U/ml, and plasma collected with 14 and 26 U/ml heparin were assayed for binding inhibitory activity. In each case, plasma or serum was incubated as described in Fig. 1, cells were washed, and ^{125}I -insulin was assayed as described in MATERIALS AND METHODS. Data are means of 2 experiments.

fetal bovine serum for the IM-9 and EBVT-1 cell lines. Male Sprague-Dawley rats (100–110 g) were from Charles River (Boston, MA).

Binding assays. Cell-binding assays for Fao, EBVT-1, IM-9, adipocytes, human monocytes (8), and erythrocytes (9) were performed as previously described except for the following modification. In all cases, except for Fao cells, the cells were washed twice with PBS, pH 7.4, washed in their respective binding buffers, and finally aliquoted in a total assay volume of 500 μl just before the experiment. The assay tubes were then preincubated at room temperature for 1 h in the presence or absence of heparin at a final concentration of 100 U/ml, washed twice with 1 ml PBS (pH 7.4 at room temperature), and resuspended in binding buffer with or without unlabeled insulin at a concentration of 100 $\mu\text{g}/\text{ml}$ to determine nonspecific binding. ^{125}I -insulin (0.1–0.2 ng/ml) was added, and the assay was incubated at 15°C for 90

min. The reaction was terminated by taking duplicate 200- μl aliquots and separating the cells by centrifugation in a Beckman B microfuge over prechilled assay buffer. The pellets were excised and counted in a Tracor Analytic 1290 γ -counter.

^{125}I -insulin binding to Fao cells was carried out on cells that had been replicate plated and grown to confluence in NUNC six-well tissue culture plates. The cells were preincubated for 1 h at room temperature \pm 100 U/ml heparin in binding buffer, washed twice with 3–4 ml PBS, pH 7.4, then incubated at 15°C for 3 h with ^{125}I -insulin with or without unlabeled insulin. The cells were again washed three times with 3–4 ml chilled PBS, solubilized in 1 ml 0.1% sodium dodecyl sulfate (SDS), and counted.

Insulin binding to solubilized insulin receptor. Solubilized partially purified insulin receptor from Fao cells was prepared as previously described (10). To measure insulin binding (11), solubilized insulin receptor (5 μg) was incubated with ^{125}I -insulin (0.1–0.2 ng/ml) in a buffer containing 100 mM HEPES, pH 8.0, 120 mM NaCl, and 2% bovine serum albumin (BSA) in the presence of unlabeled insulin and heparin (1–100 U/ml). The final assay volume was 0.2 ml. Incubation was carried out at 4°C for 16 h. The receptor was then precipitated by addition of 0.3 ml polyethylene glycol (25% wt/vol) and 0.1 ml γ -globulin (3 mg/ml). After incubation for 10 min at 4°C, the samples were centrifuged in a Beckman microfuge, washed with 12.5% polyethylene glycol, and the pellet counted for radioactivity.

Glucose oxidation bioassay. Glucose oxidation was determined by the method of Rodbell (12). Briefly, epididymal fat pads were excised from decapitated male Sprague-Dawley rats (100–110 g), placed in 3 ml of warm Krebs-Ringer bicarbonate buffer plus 2% BSA (KRB-A) containing 6 mg collagenase, gassed with 5% $\text{CO}_2/95\%$ O_2 , and placed in a gently rotating 37°C water bath for 50 min. The contents of

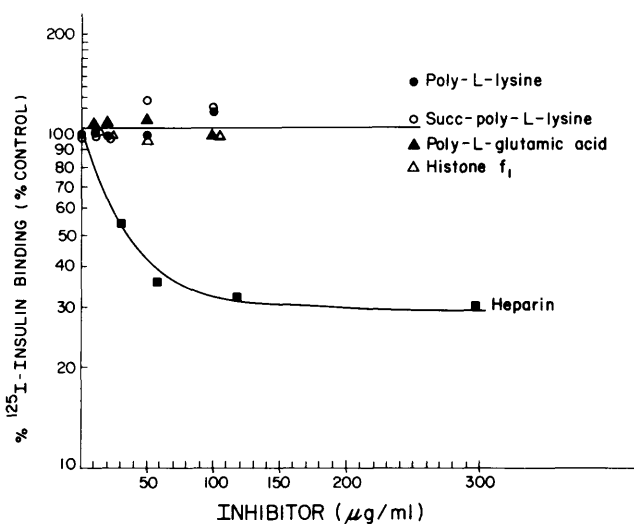


FIG. 2. Effect of polycations and polyanions on insulin binding to IM-9 lymphocytes. IM-9 lymphocytes (14.6 cells/ml) were incubated with heparin, poly-L-glutamic acid, poly-L-lysine, histone, and succinylated poly-L-lysine at indicated concentrations at 22°C for 1 h in lymphocyte-binding buffer. Cells were then washed as described in MATERIALS AND METHODS, and ^{125}I -insulin binding was assessed. Data are expressed as percent ^{125}I -insulin bound as function of untreated controls and are means of 2 experiments, each in duplicate.

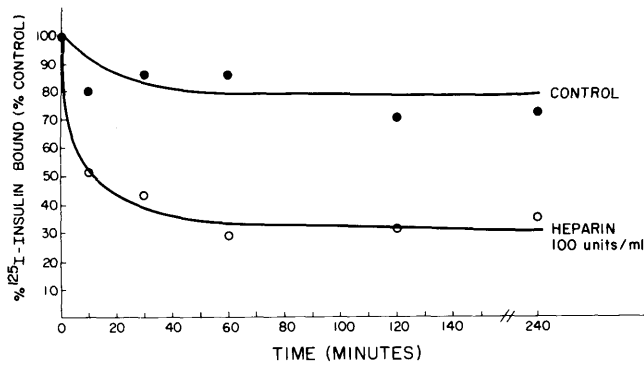


FIG. 3. Time course of heparin effect on IM-9 lymphocytes at 22°C. IM-9 lymphocytes were incubated at 22°C for different periods with 100 U/ml heparin in serum- and plasma-free buffer, extensively washed, and insulin binding measured. Data are expressed as ^{125}I -insulin binding (percent control) as function of duration of preincubation.

the 30-ml plastic digestion flask were filtered through silk mesh size 12xx, and the fat cells were washed three times with 10 ml warm KRB-A. Fat cells (100 μl of the suspension) were added to 30-ml polyethylene bottles that contained 800 μl incubation media [0.005% dextrose and 0.001% uniformly labeled [^{14}C]glucose (14.4 mCi/mmol) in KRB-A] with or without insulin. After gassing with 5% CO_2 /95% O_2 , the vials were capped and incubated at 37°C for 60 min. CO_2 was collected on filter paper treated with hyamine hydroxide and suspended in hanging wells after the incubation medium was acidified with 0.2 N H_2SO_4 . The filter paper was then placed in 15 ml scintillation fluid and counted in an LKB liquid scintillation counter.

Receptor autophosphorylation. Autophosphorylation of the β -subunit of the insulin receptor was assessed by standard in vitro techniques (10,13). Solubilized Fao insulin receptor was preincubated for 1 h at room temperature with increasing concentrations of insulin in a buffer containing 50 mM Hepes, 5 mM Mn^{2+} , and 5 mM Mg^{2+} in the absence or presence of 100 U/ml heparin. After preincubation, the phosphorylation reaction was initiated by adding 85 $\mu\text{Ci}/\text{tube}$ of [γ - ^{32}P]ATP and 1 mM cold ATP for 10 min at 22°C. The reaction was stopped by boiling a 70- μl aliquot for 3 min in Laemmli buffer under reducing conditions (100 mM dithiothreitol), which was then submitted to SDS-polyacrylamide gel electrophoresis and autoradiography.

RESULTS

Effect of heparin on insulin binding to lymphocytes.

Sera of some patients with insulin resistance contain antireceptor antibodies that inhibit insulin binding to receptors on normal cells (14,15). In the most frequently used assay for antireceptor antibodies, IM-9 lymphocytes are preincubated for 1 h at room temperature with increasing dilutions of normal or test serum, extensively washed to remove substances not tightly bound to the cell, and bound insulin is measured. Such an assay is shown in Fig. 1. Preincubation of cells with serum B-9 (in some publications, this serum has been referred to as B-8) (16) containing antireceptor antibody resulted in a dose-dependent inhibition of insulin binding with 50% effect occurring at a 1:300 dilution (Fig. 1, top). In contrast, preincubation with normal serum had no effect even up to dilutions of 1:2.

To determine the effect of various anticoagulants on insulin binding, IM-9 lymphocytes were incubated with plasma and serum from a normal individual collected under various conditions (Fig. 1, bottom). Preincubation of cells with heparinized plasma resulted in an inhibition of insulin binding. Even after the cells had been washed extensively, insulin binding was inhibited by 70 and 50% by dilutions of heparinized normal plasma of 1:2 and 1:10, respectively. When the concentration of heparin used for anticoagulation was decreased, the inhibition of binding also decreased. Serum to which heparin had been added produced a similar effect. In contrast, plasma anticoagulated with EDTA had no effect on insulin binding.

Characterization of heparin effect. When IM-9 lymphocytes were assayed with increasing concentrations of heparin diluted in binding buffer, a concentration-dependent inhibition of insulin binding was observed (Fig. 2). Some inhibition occurred with concentrations of 33 $\mu\text{g}/\text{ml}$ (5 U/ml), and inhibition was maximal at a concentration of 333 $\mu\text{g}/\text{ml}$ (50 U/ml). At this concentration, binding was reduced by 70%. The inhibitory effect of heparin was specific. Other polyanions

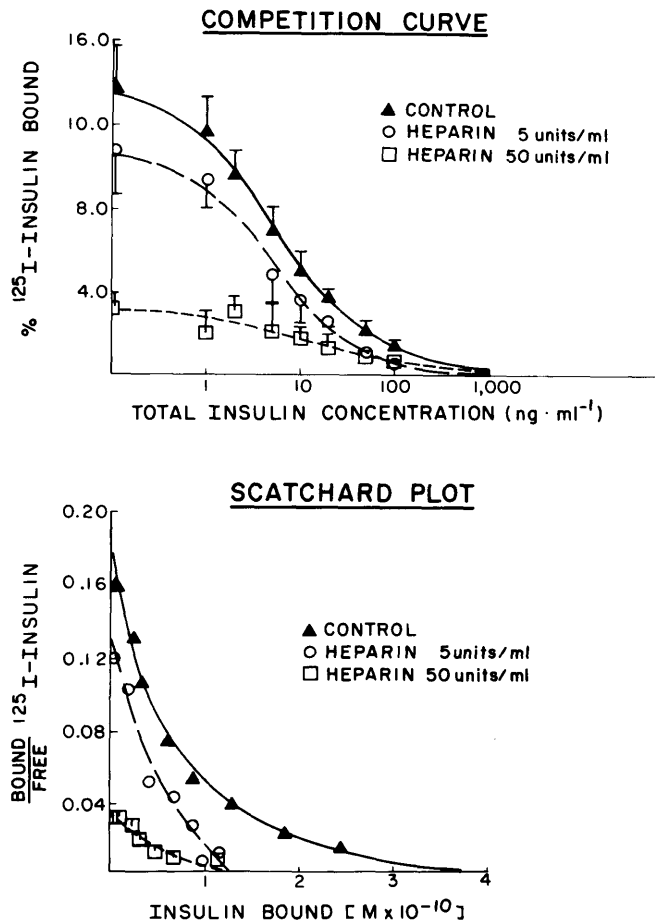


FIG. 4. Effect of heparin on insulin binding to IM-9 lymphocytes. Top: IM-9 lymphocytes (14.6×10^6 cells) were incubated with ^{125}I -insulin (10^{-10} M) and increasing concentrations of unlabeled insulin in presence or absence of 5–50 U/ml heparin. After 60 min at 22°C, percent insulin bound to cells was determined as described in MATERIALS AND METHODS. Data are means of 3 experiments. Bottom: data from upper panel are replotted by Scatchard method (17). Best-fit curves were determined with M-lab program and negative cooperative model for insulin binding (19).

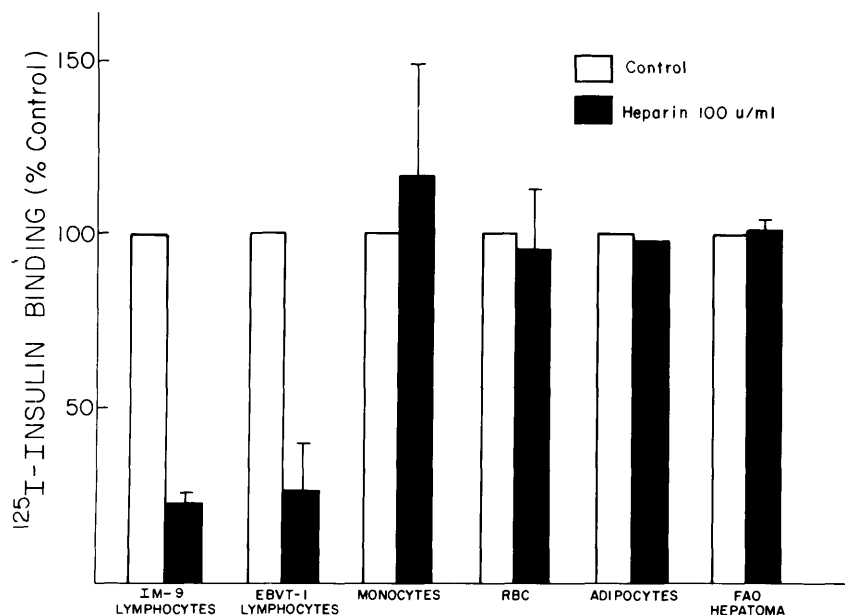


FIG. 5. Effect of heparin on ^{125}I -insulin binding to various cell types. Cells were preincubated for 1 h at 22°C with buffer or buffer containing 100 U/ml heparin, after which all cells, except adipocytes, were washed twice with PBS and then resuspended in binding buffer. ^{125}I -insulin (10^{-10} M) was then added, and insulin binding was measured. Nonspecific binding was estimated by conducting parallel experiments in presence of 10^{-6} M unlabeled insulin and was not affected by heparin. Shaded bar represents specific insulin binding after heparin treatment when expressed as percent of control. Error bars represent SE (except for adipocytes) obtained from 2 or 3 identical experiments done in duplicate.

(e.g., poly-L-glutamic acid), other polycations (e.g., poly-L-lysine and histone), and a mixed polyanion-cation succinylated poly-L-lysine produced little or no change in insulin binding (Fig. 2).

The time course of the heparin effect on insulin binding is shown in Fig. 3. The effect of heparin was very rapid; with 100 U/ml heparin, insulin binding was inhibited by 50% within 10 min. This increased to a maximum of 70% inhibition by 60 min, after which it remained stable for up to 4 h of incubation.

To further clarify the mechanism of heparin effect on IM-9 lymphocytes, competition curves for insulin binding were generated in the absence and presence of heparin (Fig. 4). Heparin inhibited tracer ^{125}I -insulin binding by 27 and 78.5% at concentrations of 5 and 50 U/ml, respectively. Competition curves with unlabeled insulin revealed a rightward shift with an increase in the concentration of insulin that produced a 50% inhibition of insulin binding from 3 to 4 and 15 ng/ml as the concentration of heparin was increased, suggesting a change in receptor affinity. Scatchard analysis (17), with a negatively cooperative model (18) and statistical curve fitting on an M-lab program (19), suggested that the low concentration of heparin produced a decrease in available receptor number, whereas the high concentration also lowered receptor affinity (Fig. 4, *bottom*). When the data were analyzed with a two-site model (20), the low concentration of heparin decreased binding by decreasing the available number of low-affinity sites, whereas the high concentration decreased the available number of both high- and low-affinity binding sites (data not shown).

The effect of heparin on insulin binding to various cell types is shown in Fig. 5. Heparin (100 U/ml) inhibited insulin binding to IM-9 lymphocytes by 60–70% and produced a similar decrease in binding in another lymphocyte cell line derived by Epstein-Barr virus transformation of normal human lymphocytes (EBVT-1) (6). Heparin had no significant effect on insulin binding to freshly isolated human monocytes, red blood cells, freshly isolated rat adipocytes, or cultured rat hepatoma cell line Fao.

Heparin effects on bioassays and on soluble receptor.

To test the effect of heparin on insulin action, isolated adipocytes were incubated with heparin, and oxidation of basal and insulin-stimulated glucose was assayed (Fig. 6). In isolated rat adipocytes, heparin caused a dose-dependent inhibition of insulin-stimulated glucose oxidation and, to a lesser extent, basal glucose oxidation. Similar to the effects on lymphocyte binding, significant inhibition was observed with heparin concentrations as low as 10 U/ml, and maximal inhibition was observed at 100 U/ml.

The effect of heparin was also studied with insulin receptors solubilized from Fao cells (Fig. 7). Even though the intact Fao cell line showed no inhibition of insulin binding with heparin, a solubilized and partially purified insulin-receptor preparation from the same cell line showed a dose-dependent inhibition of insulin binding in response to heparin. Fifty-percent inhibition of binding occurred at a heparin concentration of 100 U/ml. In addition, heparin at a concentration of 100 U/ml inhibited insulin-stimulated autophosphorylation by 30% in the same insulin-receptor preparation and caused a rightward shift in the dose-response curve (Fig. 8).

DISCUSSION

Aside from its role as an anticoagulant, heparin has been shown to interact with several different proteins and to alter their function (1–4,21). In this study, we demonstrate that heparin is a potent inhibitor of insulin binding to lymphoid cells. Heparin also inhibited insulin binding and phosphorylation to the solubilized insulin receptor, suggesting that heparin interacts directly with the insulin receptor. The exact mechanism of these heparin effects is unclear. Heparin probably interacts with the insulin receptor through ionic or electrostatic interactions that in turn result in a decrease in the affinity of the receptor for insulin.

Of the intact cell types tested, only cultured lymphoid cells exhibited an inhibition of insulin binding by heparin, possibly suggesting that heparin was not interacting directly with the receptor. However, after solubilization of the receptor from Fao cells the inhibitory effect of heparin appeared. Thus,

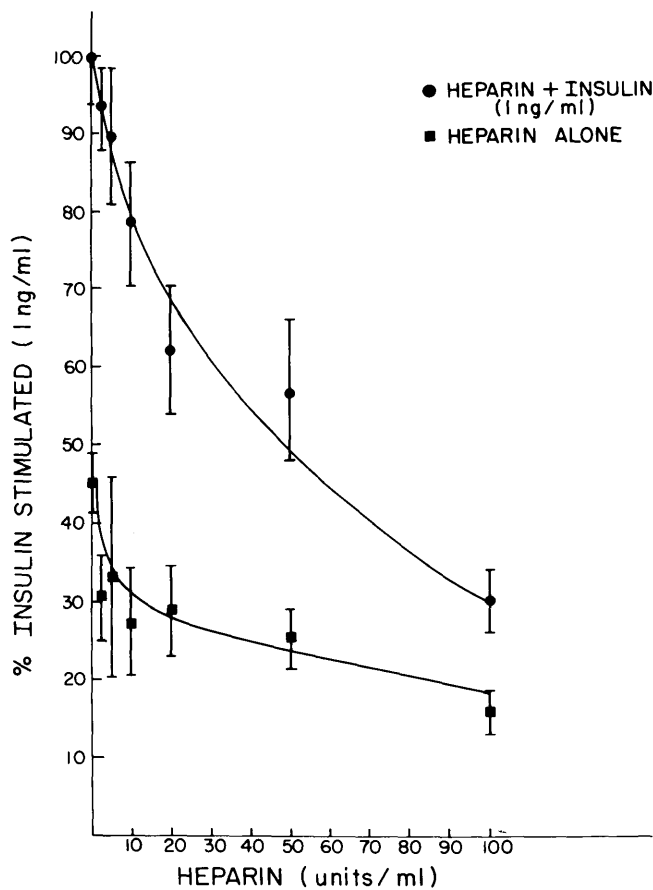


FIG. 6. Effect of heparin on glucose oxidation in isolated adipocytes. Increasing concentrations of heparin were added to isolated adipocytes, after which glucose oxidation was assessed with [^{14}C]glucose and no insulin (basal) or 1 ng/ml insulin. $^{14}\text{CO}_2$ was trapped on hyamine-soaked filter paper and counted in scintillation counter. Data are expressed as the percentage of counts found in insulin-stimulated untreated control cells. Error bars represent SE of 4 experiments.

heparin can probably interact directly with the receptor, but the domain of interaction may not be exposed in receptors in all cell membranes. Heparin also produced variable effects in the different bioactivities tested. In Fao cells, heparin had no effect on insulin binding (Fig. 5) or on tyrosine aminotransferase activity in the intact cell (data not shown). In the solubilized Fao-receptor preparation, heparin not only inhibited insulin binding but also inhibited autophosphorylation of the β -subunit of the receptor. The latter may be secondary to the inhibition of binding, because the inhibition in both cases was of a similar magnitude. With 100 U/ml heparin, insulin-stimulated phosphorylation was inhibited by 30%. These data suggest that heparin may interact differently with insulin receptors of different cell types or that the insulin receptor may have some structural differences in different cell types. Some studies have shown that insulin receptors in various tissues differ in their response to reducing agents (22,23), and even polyclonal antireceptor antibodies are not equally active in inhibiting insulin binding in all tissues (24).

Another interesting observation was that in freshly isolated adipocytes, heparin did not inhibit insulin binding but did inhibit basal and insulin-stimulated glucose oxidation. The

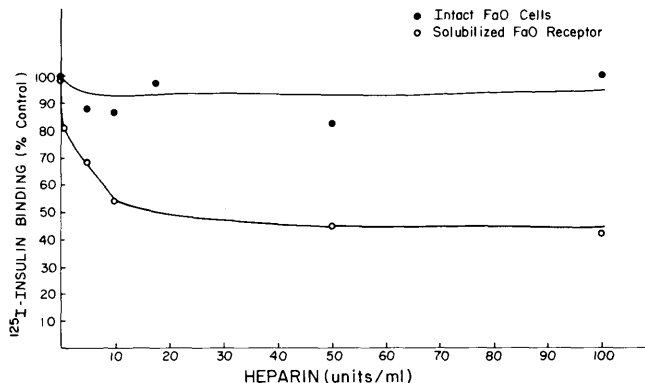


FIG. 7. Comparison of heparin effect on intact and solubilized insulin receptor from Fao cells. Solubilized and partially purified insulin-receptor preparation from Fao cell line was prepared as described in MATERIALS AND METHODS. ^{125}I -insulin binding to soluble receptor and intact cells was assessed in presence of various concentrations of heparin. Data for each are expressed as percent of untreated control and are means of 2 experiments. Differences between intact cells and soluble receptor for 10–100 U/ml heparin are significant ($P < .001$).

effect of heparin was greater in insulin-stimulated glucose oxidation, suggesting a possible effect on receptor signal transduction or a direct effect on the glucose-transport protein.

There are several practical implications of this study. First, plasma collected with heparin will produce false-positive results in antireceptor antibody assays. This could especially be a problem if different amounts of heparin were used in different samples, because this would result in the appearance of changing titers. To avoid these problems, assays for antireceptor antibody should be performed with serum or a cell type other than cultured lymphoblasts. Because the effect of heparin is greatest on lymphoid tissues, however, it is possible that no clinical effect would be observed. Furthermore, this effect may occur when heparin is given clin-

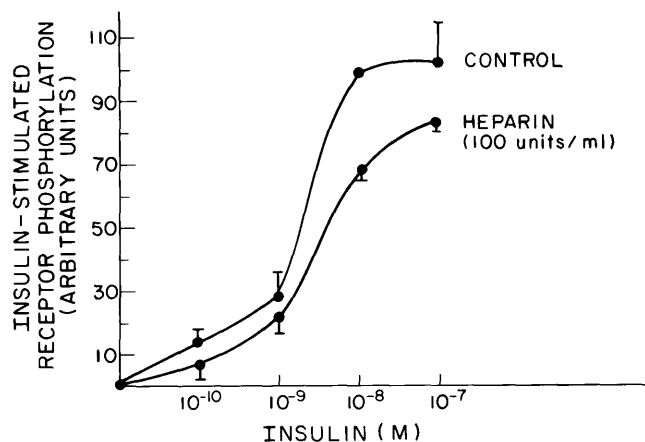


FIG. 8. Effect of heparin on insulin receptor autophosphorylation. After 1 h preincubation with indicated concentrations of insulin, solubilized Fao insulin receptor (4 μg) was incubated for 10 min at 22°C with [γ - ^{32}P]ATP. β -Subunit was separated by PAGE, located by autoradiography, excised, and radioactivity was determined by scintillation counting. Data for control are means \pm SE of 3 experiments and for heparin-treated cells are means \pm SE of 2 experiments, which are expressed as percent [γ - ^{32}P]ATP incorporation into 95,000-M, subunit of insulin receptor with 10^{-8} M insulin as 100%. Difference is significant at 10^{-8} M insulin concentration only.

ically as an anticoagulant. Data obtained from rats given low-dose heparin therapy indicate that the heparin concentration in plasma may remain at ≥ 10 U/ml (25). This concentration inhibits insulin binding in IM-9 cells by 50%. Because the heparin effect is very slowly reversible, even transient increases in the plasma level may produce sustained changes. Whether this will result in alterations in insulin action and glucose homeostasis in patients on anticoagulants needs careful study. Finally, note that heparin has been used to isolate and purify many proteins (1). Studies are underway in our laboratory to determine if the insulin receptor can be added to this list.

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REFERENCES

1. Farooqui AA: Purification of enzymes by heparin-sepharose affinity chromatography. *J Chromatogr* 184:335-45, 1980
2. Brown MS, Deuel TF, Basu SK, Goldstein JL: Inhibition of the binding of low-density lipoprotein to its cell surface receptor in human fibroblasts by positively charged proteins. *J Supramol Struct Cell Biochem* 8:223-34, 1978
3. Hubbard JR, Kalimi M: Inactivation of hepatic glucocorticoid receptors by heparin. *Biochim Biophys Acta* 755:363-68, 1983
4. Molinari AM, Medici N, Moncharmont G, Puca GA: Estradiol receptor of calf uterus. Interaction with heparin-agarose and purification. *Proc Natl Acad Sci USA* 74:4886-90, 1977
5. Gavin JR III, Gorden P, Roth I, Archer JA, Buell DH: Characteristics of the human lymphocyte insulin receptor. *J Biol Chem* 248:2202-207, 1973
6. Taylor SI, Underhill LH, Hedo JA, Roth J, Serrano-Rios M, and Blizzard RM: Decreased insulin binding to cultured cells from a patient with the Rabson-Mendenhall syndrome. Dichotomy between studies with cultured lymphocytes and cultured fibroblasts. *J Clin Endocrinol Metab* 56:856-61, 1983
7. Crettaz M, Kahn CR: Insulin receptor regulation and desensitization in rat hepatoma cells. *Diabetes* 33:477-85, 1984
8. Wachslicht-Rodbard H, Gross HA, Rodbard P, Ebert MH, Roth J: Increased insulin binding to erythrocytes in anorexia nervosa. *N Engl J Med* 300:882-87, 1979
9. Gambhir KK, Archer JA, Bradley CJ: Characteristics of human erythrocyte insulin receptors. *Diabetes* 27:701, 1978
10. White MF, Haring HH, Kasuga M, Kahn CR: Kinetic properties and sites of autophosphorylation of the partially purified insulin receptor from hepatoma cells. *J Biol Chem* 259:255-64, 1984
11. Taylor S, Hedo JH, Underhill LH, Kasuga M, Eldus MJ, Roth J: Extreme insulin resistance in association with abnormally high binding affinity of insulin receptors from a patient with leprechaunism. Evidence for a defect intrinsic to the receptor. *J Clin Endocrinol Metab* 55:1108-13, 1982
12. Rodbell M: Metabolism of isolated fat cells. Effects of hormones on glucose metabolism and lipolysis. *J Biol Chem* 239:375-80, 1964
13. Kasuga M, White MF, Kahn CR: Phosphorylation of the insulin receptor in cultured hepatoma cells and a solubilized system. *Methods Enzymol* 109:609-21, 1985
14. Flier JS, Kahn CR, Jarrett DB, Roth J: Autoantibodies to the insulin receptor. Effect on the insulin-receptor interaction in IM-9 lymphocytes. *J Clin Invest* 60:784-94, 1977
15. Kahn CR, Baird KL, Flier JS, Jarrett DB: Effect of autoantibodies to the insulin receptor on isolated adipocytes: studies of insulin binding and insulin action. *J Clin Invest* 60:1094-06, 1977
16. Kasuga M, Sasaki N, Kahn CR, Nissley SP, Rechler MM: Antireceptor antibodies as probes of insulin-like growth factor receptor structure. *J Clin Invest* 72:1459-69, 1983
17. Scatchard G: The attraction of proteins for small molecules and ions. *Ann NY Acad Sci* 51:660, 1949
18. Grigorescu F, Flier JS, Kahn CR: Characterization of binding and phosphorylation defects of erythrocyte insulin receptors in the type A syndrome of insulin resistance. *J Clin Endocrinol Metab*. In press
19. Knott GD: MLAB-A mathematical modeling tool. *Comput Programs Biomed* 10:271-80, 1979
20. Feldman HA: Mathematical theory of complex ligand binding systems at equilibrium: some methods for parameters fitting. *Anal Biochem* 48:317-38, 1972
21. Jaques LB: Heparins—anionic polyelectrolyte drugs. *Pharmacol Rev* 31:99-166, 1980
22. Jacobs S, Cuatrecasas P: Disulfide reduction converts the insulin receptor of human placenta to a low affinity form. *J Clin Invest* 66:1424-27, 1980
23. Schweitzer JB, Smith RM, Jarrett L: Differences in the organizational structure of the insulin receptor of rat adipocyte and liver plasma membranes. The role of disulfide bonds. *Proc Natl Acad Sci USA* 77:4692-96, 1980
24. Muggeo M, Ginsberg BH, Roth J, Neville DM Jr, DeMeyts P, Kahn CR: The insulin receptor in vertebrates is functionally more conserved during evolution than insulin itself. *Endocrinology* 104:1393-402, 1979
25. Jacques LB: "Low dose heparin"—significance of subcutaneous injection. *Thromb Res* 9:205-208, 1976