Virus-induced Decrease of Insulin Receptors in Cultured Human Cells

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ABSTRACT Viral infections may produce abnormalities in carbohydrate metabolism in normal subjects and profound changes in glucose homeostasis in insulin-dependent diabetics. Using an in vitro radioreceptor assay with ^{125}I-labeled insulin and human-amnion (WISH) cells, the effect of viral infections on insulin receptors was examined. Both herpes simplex virus and vesicular stomatitis virus produced a 50% decrease in insulin binding. There was no evidence that this decrease was due to degradation of insulin. On quantitative analysis, this decrease in binding was found to be the result of a decrease in receptor concentration with no change in receptor affinity. The decrease in receptors occurred between 4 and 12 h, at the time viral antigens were being inserted into the plasma membrane of infected cells. Because the $t_{1/2}$ of insulin receptors in uninfected cells was between 14 and 24 h, the decrease in insulin receptors cannot be explained solely by virus-induced shut-off of macromolecular synthesis. Moreover, viruses such as encephalomyocarditis that do not insert new antigens into the plasma membrane, did not cause changes in the number of insulin receptors. The most likely explanation is that virus-induced changes in the plasma membrane altered or displaced insulin receptors. It is concluded that the insulin receptor assay is a sensitive and quantitative method for studying the effect of viral infections on cell membranes. These data also suggest that abnormalities in glucose metabolism associated with some viral infections may be due, in part, to changes in the concentration of insulin receptors.

INTRODUCTION

Many bacterial and viral infections can cause a deterioration of carbohydrate homeostasis (1–3). This is most prominent in the diabetic where even mild infection is likely to cause increasing insulin requirements in conjunction with increased hyperglycemia, glycosuria, and acidosis (3). Changes in glucose tolerance may also occur in normal individuals during or immediately following a variety of infections (1, 2, 4). Infections may produce these changes in several ways. At the level of the beta cell, the infection may act by altering synthesis and secretion of insulin (5). In addition, it is well recognized that infection produces fever and stress, increased catabolic state, or increased secretion of corticosteroid and growth hormone, all of which may influence the secretion of insulin from beta cells or its action on peripheral target cells (6, 7). At the level of the target cell, infections might be responsible either directly or indirectly for changes in insulin receptor number or affinity, or for changes in any one of a number of metabolic pathways required for insulin action. In the present study, we investigated insulin receptor alterations in cultured human cells infected with a variety of viruses to assess the possible contribution of such alterations to the insulin resistance accompanying infection. These studies demonstrate that at least two viruses, herpes simplex virus (HSV)[1] and vesicular stomatitis virus (VSV), can produce changes in numbers of insulin receptors.

METHODS

Buffer and media. Both Eagle's minimum essential medium (MEM) and RPMI 1640 medium were supplemented with penicillin (50 U/ml), streptomycin (50 μg/ml), fungizone (0.125 μg/ml), and 2 or 10% fetal bovine serum (FBS) heat-inactivated at 56°C for 30 min.

The buffer for the insulin binding assay was 100 mM Hepes (pH 7.8 or 8.0) containing 120 mM NaCl, 1.2 mM $MgSO_4$,

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[1] *Abbreviations used in this paper*: EMC, encephalomyocarditis; FBS, fetal bovine serum; HSV, herpes simplex virus; MEM, Eagle's minimum essential medium; PBS, phosphate-buffered saline; VSV, vesicular stomatitis virus; WISH, human amnion cell line.

2.5 mM KCl, 15 mM $NaC_2H_3O_2$, 10 mM glucose, 1 mM EDTA, and 10 mg/ml of bovine serum albumin (8).

Chemicals. Porcine insulin was purchased from Elanco Products Co., Indianapolis, Ind. ^{125}I-insulin was prepared at specific activity of 100–200 μCi/μg (~0.5 atoms of iodine per insulin molecule) by the methods previously described (9). [^{3}H]leucine (45.7 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. Collagenase (type I, 125–250 U/mg), hyaluronidase (type IV, 750 U/mg), cycloheximide, and puromycin were purchased from Sigma Chemical Company, St. Louis, Mo.

Interferon. Human interferon (10^4 U/ml) was derived from Namalva lymphocytes stimulated by Newcastle disease virus (10), and was a gift from Dr. M. Smith, National Institute of Arthritis, Metabolism, and Digestive Diseases.

Cells. WISH cells (human amnion cell line), and Vero cells were grown in MEM supplemented with 10% FBS. IM-9 (human lymphocyte) cell line (11) was grown in RPMI 1640 supplemented with 10% FBS. Cell viability was determined at the beginning of each insulin binding experiment by the trypan blue exclusion method (12).

Viruses and infection of cells. HSV (F strain) VSV (Indiana strain), encephalomyocarditis virus (EMC, M-variant), Sindbis virus (Egyptian strain), and measles virus (Eli Lilly strain [Eli Lilly & Co., Indianapolis, Ind.]) were used in these studies. Measles virus was propagated in Vero cells, while all other viruses were propagated in WISH cells. HSV and measles virus infectivity titrations were performed in Vero cells, whereas infectivity titrations for the other viruses were performed in WISH cells. HSV and VSV titers were expressed in plaque-forming units; EMC, Sindbis, and measles virus titers were expressed in tissue culture infectious doses ($TCID_{50}$). In certain experiments, HSV (4×10^8 plaque-forming units/ml) was completely inactivated by exposure to ultraviolet light (UV) for 10 min at a distance of 15 cm.

To study the effect of viral infection on insulin binding, viruses were allowed to absorb to confluent monolayers of WISH cells in 75-cm^2 flasks (2×10^7 cells) for 60–90 min at 37°C in a humidified atmosphere containing 5% CO_2. Monolayers were then washed with Ca^{++} and Mg^{++} free phosphate-buffered saline (PBS), refed with 10 ml of fresh MEM containing 2% FBS, and incubated at 37°C. At various times after virus infection, cells were incubated for about 30 min at 37°C with Ca^{++} and Mg^{++} free PBS containing collagenase (1 mg/ml) and hyaluronidase (0.5 mg/ml) (13). The detached cells were washed with Ca^{++} and Mg^{++} free PBS twice, and were used for insulin binding experiments. Preliminary studies showed that treatment of WISH cells and IM-9 lymphocytes with five times higher enzyme concentrations for up to 90 min did not modify the properties of their insulin receptors (data not shown).

Insulin receptor assay. Cells were mixed with ^{125}I-labeled insulin in the presence or absence of unlabeled insulin (32 pM to 1.8 μM) in 0.5 ml of Hepes buffer. These mixtures were incubated for up to 90 min at 15°C, pH 7.8 for IM-9 lymphocytes (14), or 23°C, pH 8.0 for other cells, unless stated otherwise. After the incubation period, duplicate 200-μl aliquots of the suspension were layered onto 200 μl of chilled Hepes buffer in 400-μl plastic microfuge tubes (15). The tubes were then centrifuged for 1 min in a Beckman microfuge B (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), the supernate was aspirated, and the radioactivity in the cell pellet was counted in a gamma counter. Total binding was the radioactivity in the cell pellet, whereas the nonspecific binding represents the radioactivity in the cell pellet in the presence of 1.8 μM unlabeled insulin. Specific binding is the difference between total and nonspecific binding. The number of receptor sites per cell (R_0) was calculated from the formula:

$$R_0 = \frac{\text{mol insulin bound/ml}}{\text{cell concentration}} \times \frac{6.03 \times 10^{23} \text{ molecules}}{\text{mol}}.$$

All experiments were performed two or more times and representative results are presented.

Degradation of hormone. Degradation of ^{125}I-insulin as judged by its precipitation with TCA was determined as described by Freychet et al. (16). After the incubation of ^{125}I-insulin with cells, the cells were pelleted, and aliquots (50 μl) of the supernates were transferred to 1 ml of chilled PBS (pH 7.2) containing 0.1% of bovine serum albumin. An equal volume of 10% TCA was immediately added, and the tubes were centrifuged at 1,200 *g* for 5 min at 4°C. Radioactivity was then counted in each precipitate and supernate and the percentage of intact hormone remaining was determined (16).

The degradation of insulin was also determined by measuring the amount of ^{125}I-insulin bound to fresh IM-9 lymphocytes after its incubation for a designated period of time (14).

Inhibition of protein synthesis. Cells (5×10^6) were incubated at 37°C with MEM or MEM containing cycloheximide 18 μM (5 μg/ml) or MEM containing puromycin 5.5 μM (3 μg/ml). At different times, the medium was removed from flasks and replaced with MEM (with cycloheximide or puromycin) containing [^{3}H]leucine (2 μCi/ml). Cultures were incubated for an additional 30 min. The cultures were harvested and treated with 10% (wt/vol) TCA. Acid-insoluble material was collected on Millipore filters (Millipore Corp., Bedford, Mass.), and measured for radioactivity.

RESULTS

Binding of ^{125}I-labeled insulin to WISH cells. The effect of time and temperature on the binding of ^{125}I-insulin to human WISH cells is illustrated in Fig. 1A.

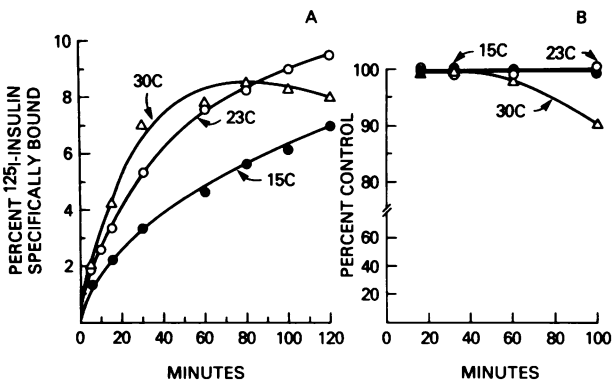

FIGURE 1 (A) Binding of ^{125}I-insulin to WISH cells as a function of time and temperature. Cells (6×10^6/ml) were incubated with 79 pM labeled insulin at 15, 23, and 30°C. At the times indicated aliquots were removed, cells were pelleted, and the amount of ^{125}I-insulin bound was determined. The supernatant fluids were used to measure insulin degradation. (B) Degradation of ^{125}I-insulin after incubation with WISH cells. Aliquots of cell-free supernatant fluids were immediately transferred into chilled plastic tubes and assayed for insulin degradation by TCA precipitation as described in Methods.

Similar to other cell types, insulin binding at 30 and 23°C was much more rapid than at 15°C. At 23 and 30°C, binding reached a maximum at 80–90 min. However, binding decreased with further incubation at 30°C. To see if this was due to degradation of insulin in this system, supernatant fluids from cells incubated with ^{125}I-labeled insulin were precipitated with TCA. The data in Fig. 1B shows that there is essentially no degradation of insulin when the cells are incubated at 15° or 23°C for up to 100 min. In contrast, ~10% of ^{125}I-insulin was degraded after 100 min at 30°C. Based on these studies, insulin binding experiments with WISH cells were performed at 23°C over a period of 90 min.

Effect of HSV infection on the binding of insulin. Confluent monolayers of WISH cells were infected with HSV and at varying times thereafter, the amount of insulin bound was determined. Between 2 and 4 h after infection, a marked decrease in the specific binding of insulin was observed (Fig. 2). These changes were maximal at 8 h at which time insulin binding was decreased by ~40%. There was no alteration of cell viability as measured by trypan blue exclusion and no increase in nonspecific binding. The effect of HSV on insulin binding, when measured 8 h after infection, was dependent on the concentration of virus used to infect the cells (data not shown). Maximal inhibition was observed with a virus to cell ratio of 100, and near maximal inhibition was achieved with a virus to cell ratio of 10. Only partial inhibition was observed at lower rates.

Effect of UV-irradiated HSV on ^{125}I-insulin binding. Cells were incubated with UV-inactivated HSV (at a virus to cell ratio of six) and at different times thereafter the binding of ^{125}I-insulin was measured. As seen in Table I, similar amounts of ^{125}I-insulin bound to uninfected cells and cells that had been incubated with UV-inactivated virus. Thus, infectious virus is needed to depress insulin binding.

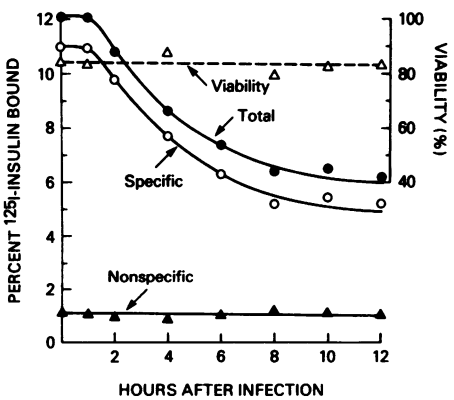

FIGURE 2 Effect of HSV infection on insulin binding. WISH cells were infected with HSV at a virus to cell ratio of six. At 2-h intervals, 1 × 10^7 cells/ml were harvested and incubated with 45 pM labeled insulin for 90 min at 23°C. Aliquots were removed, the cells were pelleted, and the amount of ^{125}I-insulin bound was determined. The supernatant fluids were saved to test for insulin degradation (Table II).

TABLE I
*Effect of UV-irradiated HSV on Insulin Binding to Cells**

Hours after inoculation of virus	% Insulin bound‡		% Viability	
	UV-HSV	Uninfected	UV-HSV	Uninfected
8	9.2	8.6	95.0	93.0
24	6.0	5.5	98.4	98.4
48	7.0	6.8	90.1	88.6
72	12.2	11.6	95.9	95.5

* WISH cells were inoculated with UV-irradiated HSV (at a virus to cell ratio of six). At various times after incubation, the cells were harvested, resuspended at a concentration of 0.8–1.0 × 10^7 cells/ml, and incubated with ^{125}I-insulin (52 pm) for 90 min at 23°C. The amount of insulin bound was measured as described in Methods.
‡ Total insulin bound less the nonspecific binding.

Degradation of insulin. Decrease in insulin binding after viral infection also might be caused by an increase in the degradation of insulin making less insulin available for cell binding. Therefore, we investigated the possibility that enzymes released from infected cells might be degrading the ^{125}I-insulin. WISH cells were infected with HSV for 8 to 10 h, incubated with ^{125}I-insulin, and the amount of insulin bound to the cells was determined. Supernatant fluids from these cultures were used to measure insulin degradation by either TCA precipitation or rebinding of insulin to fresh cells. Table II shows that supernatant fluids from infected and uninfected WISH cells contained the same amount of intact ^{125}I-insulin as evaluated by both methods. Thus, the decrease in insulin binding in infected cells is not due to degradation of insulin.

Degradation of insulin receptor. Degradation of the insulin receptor by enzymes released from infected

TABLE II
*Degradation of ^{125}I-insulin Exposed to Infected and Uninfected Cells**

Hours after infection	% Rebinding‡		% Precipitation by TCA§	
	Infected	Uninfected	Infected	Uninfected
8	29.9	31.8	96.6	95.4
10	35.1	32.0	94.6	94.4

* WISH cells were infected with HSV at a virus to cell ratio of six (Fig. 2). After 8 and 10 h of incubation, 1 × 10^7 cells/ml were harvested and incubated with 45 pm ^{125}I-insulin for 90 min at 23°C. Samples were then removed, cells were pelleted, and the amount of ^{125}I-insulin bound was determined. Supernatant fluids from pelleted cells were used to measure insulin degradation by TCA precipitation and binding of insulin to fresh cells.
‡ Part of the supernatant fluid was incubated with IM-9 lymphocytes (5 × 10^6 cells) for 90 min at 15°C and the amount of ^{125}I-insulin bound was determined. Nonspecific binding has been subtracted.
§ Part of the supernatant fluid was mixed with TCA, centrifuged, and the percentage of radioactivity in the precipitate was determined.

TABLE III

Effect of Supernatant Fluid from Virus-infected Cell Cultures on Insulin Binding to Fresh Cells

	Cells treated with supernatant fluid from		
	HSV-infected cells	VSV-infected cells	Untreated cells
		%	
Total binding	17.2	18.5	15.8
Specific binding	16.0	17.5	14.6
Nonspecific binding	1.2	1.0	1.2
Viability	86.7	88.5	86.2

WISH cells infected with HSV (at a virus to cell ratio of 20) and VSV (at a virus to cell ratio of 20) were incubated for 8 h at 37°C. Supernatant fluids from these cultures were treated with UV-light for 5 min at a distance of 15 cm, and then incubated with fresh WISH cells for 8 h at 37°C. After this incubation, 1×10^7 cells/ml were incubated with ^{125}I-insulin for 90 min at 23°C, and ^{125}I-insulin bound was determined.

cells also might cause a decrease in insulin binding. WISH cells were infected with HSV or VSV at a virus to cell ratio of 20. After 8 h, the supernatant fluids were harvested, treated with UV-light to inactivate infectious virus, and then incubated with fresh WISH cells for 8 h. The cells were then incubated with ^{125}I-insulin and the amount of insulin bound was determined. Approximately the same amount of insulin bound to untreated cells as to cells that had been incubated with supernatant fluids from infected cells, suggesting that the insulin receptor had not been degraded by enzymes released into the media by infected cells (Table III).

Effect of virus infection on receptor affinity and receptor number. To see whether lower binding of ^{125}I-insulin was due to a change in receptor concentration or receptor affinity, cells that had been infected with HSV for 8 h were harvested and ^{125}I-insulin binding was measured in the presence of different concentrations of unlabeled insulin. As is seen in Fig. 3, the amount of labeled insulin bound to infected cells was consistently about half that bound to uninfected cells. The concentration of unlabeled insulin that produced 50% inhibition of tracer binding for infected and uninfected cells was 2 ng/ml, suggesting that the virus infection produced a decrease in receptor concentration without changing receptor affinity.

This was confirmed when the data were analyzed by the method of Scatchard (Fig. 3, insert). There was a parallel displacement of the infected cell curve to the left of the uninfected cell curve. Total receptor concentration as determined from the abscissa intercept was decreased by 44% with little or no change in receptor affinity.

Effect of other viral infections on ^{125}I-insulin binding. To study the effect of other viruses on the insulin receptor, VSV, EMC, Sindbis, and measles viruses were allowed to adsorb to confluent monolayers of WISH cells. At various times after infection, the cells were harvested and ^{125}I-insulin binding was measured. As is seen in Fig. 4, within 4 h after inoculation of cells with VSV, specific insulin binding was decreased, and was depressed by as much as 68% at the end of 11 h. Despite a marked decrease in insulin binding, there was no alteration in cell viability. As with HSV, the decrease in insulin binding was dependent on the virus

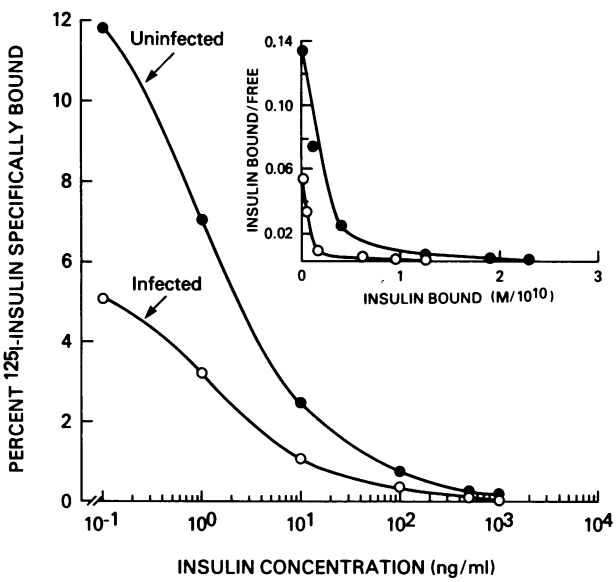

FIGURE 3 Comparison of insulin binding to HSV-infected WISH cells and uninfected WISH cells. WISH cells were infected with HSV at a virus to cell ratio of six. After 8 h of incubation, the cells were harvested and ^{125}I-insulin binding was measured. The cells (1×10^7/ml) and ^{125}I-insulin (45 pM) were incubated for 90 min at 23°C with increasing concentrations of unlabeled insulin. The insert shows the Scatchard analysis of the same data. Bound to free insulin is plotted against bound insulin.

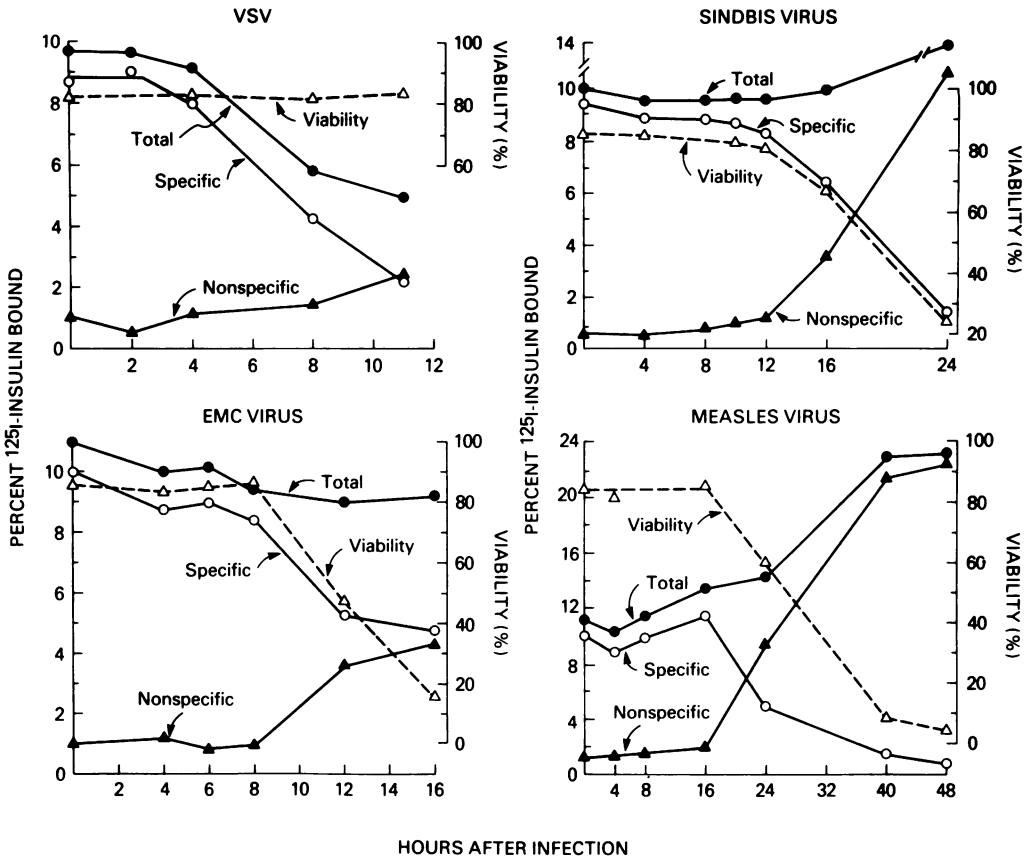

FIGURE 4 Insulin binding to virus infected cells. WISH cells were infected with VSV, Sindbis virus, EMC virus, and measles virus at a virus to cell ratio of 20, 7, 10, and 1.5, respectively. At various times thereafter, the cells were harvested. VSV infected cells (7×10^6/ml), Sindbis virus-infected cells (8×10^6/ml), EMC virus-infected cells (8×10^6/ml), and measles virus-infected cells (1×10^7/ml) were incubated with 25–80 pM labeled insulin for 90 min at 23°C, and the amount of ^{125}I-insulin bound was determined.

to cell ratio and was maximal at a ratio of 10 (data not shown).

In the case of EMC, Sindbis, and measles viruses, there was little alteration in specific binding at 8, 12, and 16 h after infection, respectively. After these times, specific binding decreased in all three cases, however, this was closely correlated with a decrease in cell viability and with an increase in nonspecific binding of insulin. There also was an increase of total binding in the case of Sindbis and measles virus-infected cells, while the total binding remained relatively constant with EMC virus. In a separate experiment, the capacity of these viruses to grow in WISH cells was determined (data not shown). Significant increases in the yield of infectious HSV, VSV, Sindbis virus, and EMC virus could be detected within 8 to 10 h. Progeny measles virus was not detected until 14–16 h after inoculation.

Effect of interferon on ^{125}I-insulin binding. To see whether the decrease in insulin receptors might be related to the induction of interferon by viruses, WISH cells were incubated with different concentrations of interferon and the binding of ^{125}I-insulin was determined. Up to 10,000 U of interferon failed to alter the binding of insulin (Table IV). Similarly, interferon induced from human leukocytes by purified protein derivative had no effect on insulin binding (data not shown).

Effect of inhibition of protein synthesis on insulin binding. In an attempt to measure the turnover of insulin receptors, WISH cells were treated with inhibitors of protein synthesis. When WISH cells were exposed to cycloheximide at 18 μM, cellular protein synthesis was depressed by 90% within 30 min and by 97% within 6 h, as estimated by incorporation of [^{3}H]leucine (Fig. 5A, insert). Viability of treated cells was the same as untreated control cells (87.5%) for up to 24 h (data not shown). Under these conditions, insulin binding was reduced in a time-dependent fashion with an estimated $t_{1/2}$ of >24 h (Fig. 5A). When WISH cells were exposed to puromycin at 5.5 μM, cellular protein

TABLE IV

Effect of Interferon on Insulin Binding to Cells

Interferon titer (U/ml)	% Specific binding	% Viability
Expt. 1		
10^3	6.7	92.0
10^2	7.3	94.1
10^1	7.9	87.6
Control	6.8	88.6
Expt. 2		
10^4	10.7	78.3
10^3	11.4	88.0
Control	11.8	85.0

WISH cells were incubated with various concentrations of type 1 interferon (lymphoblastoid). After an incubation period of 48 h (Expt. 1) or 30 h (Expt. 2), 1×10^7 cells/ml were incubated with ^{125}I-insulin for 90 min at 23°C and ^{125}I-insulin bound was determined.

synthesis was depressed to 75% within 30 min and by 99% within 12 h (Fig. 5B, insert). Viability of treated cells was the same as untreated control cells (80%) for up to 14 h and thereafter, it began to decrease (data not shown). Insulin binding decreased in a time-dependent fashion, with an estimated $t_{1/2}$ of 14 h (Fig. 5B).

DISCUSSION

In this study we used the radioreceptor assay for insulin as a means of studying the effect of viral infection on the plasma membranes of cells. This in vitro system has several advantages. The number of receptors and receptor affinity can be quantitated quickly and accurately. In addition, the assay allows one to discriminate between specific binding of insulin to the receptor and nonspecific binding secondary to cell damage. Using this assay system, we demonstrated that some viral infections affect insulin receptors. In the case of HSV and VSV, the decrease in insulin receptors occurred rapidly and appears to be due to a true decrease in receptor number, rather than an alteration in receptor affinity.

The number of insulin receptors on the surface of a cell reflects the rates of both synthesis and degradation. There are several ways in which viruses could influence insulin binding. First, viruses could shut off host cell macromolecular synthesis, thereby resulting in a decrease in the number of insulin receptors. Second, viruses could alter the number of receptors on the plasma membrane by masking or chemically modifying the receptor. Third, viruses might mobilize lysosomal enzymes that could degrade the receptor.

It is known that a number of viruses can shut off host cell macromolecular synthesis within hours after infection (17). However, at least two lines of evidence ar-

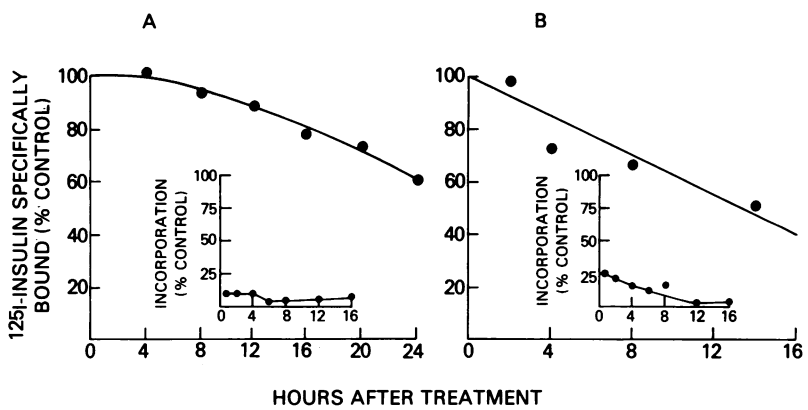

FIGURE 5 The effect of cycloheximide (A) and puromycin (B) on insulin binding to WISH cells. Cells were preincubated for various time periods with cycloheximide (18 μM) or puromycin (5.5 μM). At various times cells were harvested, washed, and incubated with ^{125}I-insulin. The specific binding of ^{125}I-insulin was measured and the results expressed as percentage of untreated control cells. Specific binding of ^{125}I-insulin to untreated control cells (1×10^7/ml) was 12%.

The inhibition of protein synthesis by cycloheximide or puromycin was measured by the incorporation of [^{3}H]leucine into acid-precipitable material. Inserts: duplicate flasks containing 5×10^6 cells were incubated at 37°C with or without cycloheximide (18 μM) or puromycin (5.5 μM) dissolved in fresh MEM. At different times thereafter, the medium was removed from the flasks and replaced with MEM (with or without cycloheximide or puromycin) containing [^{3}H]leucine (2 μCi/ml). The cultures were incubated for an additional 30 min, then harvested, treated with 10% (wt/vol) TCA, and the acid-insoluble material was collected on Millipore filters and measured for radioactivity. Incorporation of [^{3}H]leucine into untreated control cells was 3×10^4 cpm.

gue against this possibility as the only mechanism by which viruses decrease insulin receptors. First, EMC virus, which shuts off macromolecular synthesis within 2 h after infection (18), did not produce a decrease in the number of insulin receptors until well after 8 h, when there was a loss in cell viability. Second, the $t_{1/2}$ of the insulin receptor (Fig. 5) (19, 20) is between 14 and 24 h. Our data show that a decrease in receptors occurs within 2 to 4 h and is maximal at 8 to 10 h after infection with HSV or VSV.

Viruses also might decrease the number of insulin receptors by inserting viral antigens into the plasma membrane. In fact, within 2 to 4 h after infection, viral antigens are found in the plasma membranes of cells infected with HSV and VSV (21–23). This might mask, displace, or chemically modify insulin receptors. Alternatively, the infection might lead to internalization or shedding of the receptor. Such processes have been reported in tumor cell antigen systems and HLA antigen systems (24, 25).

In our experiments, infection with measles and Sindbis virus did not result in a decrease in the number of insulin receptors. These viruses insert antigens into the plasma membranes, but relatively late in the course of the infection (22, 26, 27). At this stage of the infection, cell death and nonspecific insulin binding is high, making it difficult to evaluate the specific effect of these viruses on insulin receptors. In contrast, picornaviruses do not insert new antigens into the plasma membrane (22, 28), and in our experiments EMC virus did not alter insulin receptors. Taken together, these observations support the argument that at least some viruses that insert antigens into plasma membranes can affect the number of insulin receptors. The extent of this effect is undoubtedly dependent on the amount of viral antigens inserted into the membrane, the precise site of insertion, and whether these changes occur early or late during the course of the infection.

Viruses also might cause a decrease in insulin receptors by mobilizing and releasing lysosomal enzymes from infected cells (29, 30). In our experiments, however, this seems unlikely since supernatant fluid from infected cells did not degrade insulin receptors.

Earlier experiments demonstrated that viruses can alter markers on the plasma membrane of infected cells. Human cells infected with VSV showed a 50% decrease in HLA antigens (28) and mouse cells infected with VSV, Newcastle disease virus, and vaccinia virus showed a 50–70% decrease in H-2 antigens (31–34). Both shut-off of macromolecular synthesis and insertion of viral antigens into the plasma membrane have been suggested as possible explanations for the decrease in cell surface markers (28, 31). Certain viruses also can alter the agglutinability of cells. VSV (35), Newcastle disease virus (36), vaccinia virus (37), HSV (38), and Sindbis virus (39) greatly increased the agglutinability of infected cells when exposed to concanavalin A, presumably by redistributing the concanavalin A receptor.

It is possible that products formed during certain viral infections may, in fact, be the common denominator responsible for the reduction of insulin receptors. For example, interferon is produced in response to a number of viral infections. It also can be produced by lymphocytes during the host's immune response to foreign antigens. Interferon not only inhibits viral synthesis but recently has been shown to have profound effects on cellular macromolecular synthesis, markers on the plasma membrane (e.g., H-2 and HLA) and receptors for the binding of cholera toxin, thyroid-stimulating hormone, and concanavalin A (40–44). In our study, up to 10,000 U of interferon failed to produce any significant change in the number of insulin receptors. It is known, however, that a variety of chemicals, drugs, and hormones such as corticosteroids and insulin can alter insulin receptors (7, 45, 46). Some of these factors are known to be released and to circulate in increased concentrations during viral infections. It is possible that these or other factors might contribute to insulin resistance by affecting insulin receptors.

In conclusion, our experiments show that insulin receptors can be used as a sensitive indicator to study the effect of viruses on the plasma membrane of cells. In addition, the decrease in the number of insulin receptors induced directly or indirectly by viruses might explain, in part, the increased requirement for insulin in diabetic patients during infection.

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