The Binding of Insulin to Mouse Leucocytes During Viral Infections

F. Shimizu¹, C. R. Kahn^{2*}, C. Garzelli¹, J. J. Hooks¹, and A. L. Notkins¹

¹Laboratory of Oral Medicine, National Institute of Dental Research and

²Section in Cellular and Molecular Physiology, Diabetes Branch, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA

Summary. The effect of viral infections on insulin binding in vivo was evaluated by measuring the binding of ¹²⁵I-insulin to several different tissues. We found that splenic leucocytes from mice infected with either the diabetogenic (D) or non-diabetogenic (B) variants of encephalomyocarditis virus, herpes simplex virus, or lactic dehydrogenase virus showed up to a 130% increase in insulin binding. As much as a 300% increase in the binding of ¹²⁵I-insulin to splenic leucocytes was observed in mice given bacterial lipopolysaccharide. In neither virus-infected nor lipopolysaccharide-treated mice was there any substantial change in insulin receptors on thymocytes, liver membranes, or peripheral erythrocytes. Thus, the

increased binding of insulin appears to be limited to leucocytes and does not appear to represent a generalized metabolic alteration. These experiments suggest that during infection, the binding of insulin to leucocytes, which is widely used to measure insulin receptors, may not always accurately reflect the insulin receptor status of other tissues.

Key words: Insulin binding, viral infections, encephalomyocarditis virus, herpes simplex virus, lactic dehydrogenase virus, bacterial lipopolysaccharide, murine splenic leucocytes, liver membranes.

A number of physiological and pathological factors have been shown to regulate the concentration and/or affinity of insulin receptors, as well as to alter various post-receptor steps involved in insulin action [1, 2]. Little is known, however, about the effect of viral infections on insulin binding [3], especially to leucocytes. The present experiments in vivo show that the binding of insulin to splenic leucocytes is markedly increased in animals infected with viruses or given bacterial lipopolysaccharides.

Methods

Mice and Viruses

Male SJL/J, CBA and NIH/Swiss mice (age 4–5 weeks) and several different viruses were used in these experiments: a diabetogenic variant of encephalomyocarditis (EMC) virus, designated EMC-D [4]; a non-diabetogenic variant of EMC virus, designated EMC-B [4]; herpes simplex virus (type 1, F strain); and lactic dehydrogenase virus [5]. Mice were inoculated intraperitoneally with viruses or *Escherichia coli* K 235 LPS (a gift from Dr. R. Moore, National Institutes of Health, Bethesda) and, at various times thereafter, tissues were removed and prepared for insulin binding assays.

Chemicals

Porcine insulin was purchased from Elanco Products, Indianapolis, Indiana; bovine serum albumin (BSA) from Sigma, St. Louis, Missouri; and NH₄Cl from Mallinckrodt, St. Louis, Missouri.

Preparation of Cells and Membranes

Splenic leucocytes were prepared using 155 mmol/l NH₄Cl buffer (pH 7.0) [6]. Thymocytes were prepared by passing minced thymus through a stainless steel screen (100 µm), washed twice with RPMI 1640, and then used for insulin binding. Erythrocytes were isolated from heparinized blood by three separate gradient separations [6]. Liver membranes were obtained by differential centrifugation of homogenates prepared in sucrose (0.25 mol/l) with a polytron PT-10/30 homogenizer (Brinkmann Instruments, Westbury, New York), as described by Cuatrecasas [7].

Leucocyte Subpopulations in Spleen

The percentage of B cells (Ig⁺) and T cells (Thy 1.2⁺) was determined by standard complement-dependent cytotoxicity (i.e., trypan blue exclusion) using rabbit anti-mouse immunoglobulin serum or anti-mouse Thy 1.2 antibody, respectively. The percentage of macrophages was determined by measuring the number of esterase-positive cells or, in some experiments, the number of cells that took up latex particles. In control animals, the percentage of B cells, T cells and macrophages was 38.3 ± 2.5%, 39.0 ± 4.3% and 8.9 ± 1.5%, respectively.

* Current Address: Joslin Diabetes Center, 1 Joslin Place, Boston, MA, 02215, USA

Fig. 1 A–F. Effects of viral infections and lipopolysaccharide administration on ^{125}I -insulin binding to splenic leucocytes. On the days indicated after IP inoculation of virus or lipopolysaccharide, splenic leucocytes ($6\text{--}8 \times 10^7/\text{ml}$) from two mice were pooled and incubated with porcine ^{125}I -insulin ($100\text{--}200 \mu\text{Ci}/\mu\text{g}$) in the presence or absence of $1.8 \mu\text{mol/l}$ unlabelled insulin for 120 min at 15°C . The results are expressed as a percentage increase in specific binding of ^{125}I -insulin above controls ($\frac{\text{infected-control}}{\text{control}} \times 100$). Each point is the mean $\pm$ SEM of six mice. Mean specific binding from untreated control mice (8×10^7 cells/ml) was $2.0 \pm 0.07\%$ in SJL/J mice, $2.7 \pm 0.09\%$ in CBA mice, and $2.7 \pm 0.1\%$ in NIH/Swiss mice. There was no significant change in non-specific binding or cell viability when the treated and untreated control mice were compared. (+) $p < 0.05$; (*) $p < 0.01$; (**) $p < 0.002$ shows significant difference in ^{125}I -insulin binding. Individual panels: (A) Diabetogenic EMC-D virus (5×10^5 plaque-forming units, PFU), inoculated into diabetes-prone SJL/J mice. (B) EMC-D virus (1×10^5 PFU) inoculated into diabetes-resistant CBA mice. (C) Non-diabetogenic EMC-B virus (5×10^5 PFU) inoculated into SJL/J mice. (D) Herpes simplex virus (F strain; 1×10^6 PFU) inoculated into NIH/Swiss mice. (E) Lactic dehydrogenase virus (P 33; 10 infectious dose₅₀) inoculated into SJL/J mice. (F) Lipopolysaccharide ($70 \mu\text{g}$; < 0.5 lethal dose₅₀) inoculated into SJL/J mice.

Insulin Receptor Assay

^{125}I -insulin (spec. act.: $100\text{--}200 \mu\text{Ci}/\mu\text{g}$) was prepared as described previously [8]. The binding of ^{125}I -insulin to splenic leucocytes (50 mmol/l Hepes buffer, pH 8.0, containing 1% BSA), erythrocytes (50 mmol/l Hepes buffer, pH 8.2, containing 0.1% BSA), thymocytes (50 mmol/l Hepes buffer, pH 8.0, containing 1% BSA), and liver membranes (Krebs-Ringer phosphate buffer, pH 7.5, containing 1.5% BSA) was performed as described previously [6, 8]. Total binding represents the amount of ^{125}I -insulin bound to cell membranes in the absence of unlabelled insulin. Non-specific binding represents the amount of ^{125}I -insulin bound in the presence of $1.8 \mu\text{mol/l}$ of unlabelled insulin for splenic leucocytes, thymocytes and erythrocytes or $18 \mu\text{mol/l}$ of unlabelled insulin for liver membranes. Specific binding is the difference between total and non-specific binding. All experiments were performed two or more times and representative results are presented.

Results

When diabetes-prone SJL mice were inoculated with the diabetogenic D-variant of EMC virus [4], the binding of ^{125}I -insulin to receptors on splenic leucocytes increased (Fig. 1 A). A 50% increase in binding was apparent at 2 days and a maximal increase of 130% above

control 6 days after infection. By 10 days the binding of insulin to splenic leucocytes had returned to normal. Since it is known that the concentration of insulin receptors on cells is inversely related to the concentration of extracellular insulin [9], and since the D-variant of EMC virus produces hypoinsulinaemia [4], it was possible that the increase in insulin binding was secondary to changes in the insulin concentration in the circulation. To evaluate this possibility, we infected diabetes-resistant CBA mice [10] with the D-variant of EMC virus. Although this virus does not produce diabetes in CBA mice, the increase in binding of ^{125}I -insulin to splenic leucocytes was similar to that observed in SJL/J mice 4 days after infection and returned to normal within 10 days (Fig. 1 B).

Other viral infections also produced an increase in the binding of ^{125}I -insulin. As seen in Figure 1 C, splenic leucocytes from mice infected with the non-diabetogenic B-variant showed an increase in insulin binding. The magnitude of the increase was less than that observed with the D-variant, consistent with previous studies which showed that the non-diabetogenic B-variant replicates poorly [4]. Moreover, leucocytes from mice in-

Fig. 2A–E. Effects of viral infection and lipopolysaccharide administration on ¹²⁵I-insulin binding to thymocytes, erythrocytes, and liver membrane. Thymocytes (2×10^8 /ml, pooled from two mice), erythrocytes (2.8×10^9 /ml, pooled from two mice), and liver membrane (0.8 mg/ml, pooled from three mice) were incubated with ¹²⁵I-insulin (100–200 μ Ci/ μ g) at 15 °C for 120 min (thymocytes and erythrocytes), or 240 min (liver membrane). Each bar is the mean $\pm$ SEM of six mice. Specific binding is indicated by the bars above the baseline: (■) untreated mice; (▨) treated mice. Non-specific binding is indicated by the bars below the baseline (□). The panels show ¹²⁵I-insulin binding to: **A** Thymocytes from EMC-D virus-infected SJL/J mice (from Fig. 1A). **B** Erythrocytes from EMC-D virus-infected SJL/J mice (from Fig. 1A). **C** Liver membrane from EMC-D virus-infected SJL/J mice (from Fig. 1A). **D** Liver membrane from herpes simplex virus infected NIH/Swiss mice (from Fig. 1D). **E** Liver membrane from lipopolysaccharide inoculated SJL/J mice (from Fig. 1F)

ected with herpes simplex virus or lactic dehydrogenase virus also bound more insulin than leucocytes from uninfected mice (Fig. 1D and E).

The demonstration that leucocytes from virus-infected mice bound more insulin than controls suggested that other infectious agents or toxins might produce similar changes. When mice were inoculated with bacterial lipopolysaccharide, an even more dramatic increase in insulin binding was observed (Fig. 1F). By 4 days, insulin binding was approximately 300% above normal levels. As with the viral infections, the increase in binding was transient and returned to normal at 10 days after inoculation.

Although both viral infections and lipopolysaccharide increased the capacity of leucocytes to bind insulin, the effect seems to be limited to leucocytes and does not appear to represent a generalized metabolic alteration. As seen in Figure 2A–C, thymocytes, erythrocytes and liver membranes from mice infected with the D-variant of EMC virus showed little or no increase in the amount of ¹²⁵I-insulin bound compared with tissues from uninfected mice. Liver membranes from mice inoculated with herpes simplex virus or lipopolysaccharide also showed little, if any, change in insulin binding compared with uninoculated controls (Fig. 2D and E), although at 2 days after inoculation of lipopolysaccharide ¹²⁵I-insulin binding to liver membranes was increased. If Kupffer cells respond to lipopolysaccharide in a fashion similar to splenic leucocytes, their presence in the liver might account for the observed increase in insulin binding.

At the time of maximum increase in insulin binding (i.e., 4–6 days), the percentage of B cells, T cells and macrophages in the spleens of infected mice was determined. Animals infected with the D variant of EMC virus, lactic dehydrogenase virus, or herpes simplex virus showed no significant change as compared to uninfected controls. In mice given lipopolysaccharide, there was a 15–25% increase in macrophages and B cells.

Discussion

Receptors for insulin have been found on various cell types belonging to the immune system. Macrophages and circulating monocytes have moderate numbers of insulin receptors [11] and have proved useful for clinical studies, whereas granulocytes have only low levels of receptors [12] and quiescent lymphocytes have almost none [13, 14]. If, however, lymphocytes are stimulated with mitogens or allogeneic antigen, receptors are readily induced. Insulin receptors emerge on T lymphocytes after concanavalin A or phytohemagglutinin treatment, and during mixed lymphocyte reactions and allogeneic skin grafting [13, 14]. Insulin receptors also are induced on B lymphocytes after in vitro activation with lipopolysaccharide [14].

Many studies have shown that viruses can stimulate cells of the immune system directly on an antigen-specific basis or indirectly as a result of the release of mediators such as interferon or interleukin 2 [15–17]. Viruses

(e.g. Epstein-Barr virus) and mitogens (e.g. lipopolysaccharide) also are known to be polyclonal B cell activators [18, 19]. In the present study, the increase in insulin binding following infection was limited to leucocytes. It is known that leucocyte subsets can dynamically change during the course of viral infections. Thus, despite our measurement of B cells, T cells, and macrophages, we cannot say with absolute certainty whether the increase in insulin binding is due to an increase in the number of insulin receptors per cell, or an expansion of subsets of leucocytes bearing insulin receptors, or both.

The increase in insulin binding to splenic leucocytes in this study contrasts with the decrease in insulin binding to cultured virus-infected epitheloid cells reported earlier [3]. These two situations, however, are quite different. In the experiments with cultured epitheloid cells, the infection resulted in the insertion of viral antigens into the plasma membrane of cells which is thought to alter or displace insulin receptors [3]. This also could occur in vivo, but only at the very localized sites of infection. In the present study, the increase in insulin binding is most likely a secondary effect due to leucocyte activation [13, 14] and/or expansion of a leucocyte subset. Alternatively, virus-induced stress could lead to the release of hormones (e.g. glucocorticoids, growth hormone) [20] which, in turn, could produce alterations in insulin-receptor interaction and insulin sensitivity. Interestingly, in both experiments in vitro and in vivo, some glucocorticoids have been shown to produce an increase in insulin binding to leucocytes [21, 22], while producing no change or even a decrease in insulin binding to other tissues [23, 24].

The demonstration that viruses and lipopolysaccharides enhance the binding of insulin to leucocytes, but not liver membranes, argues that peripheral leucocytes, which are widely used to evaluate insulin receptors, may not always accurately reflect the insulin receptor status of other tissues during infection. In addition, the present study points to the possibility that a variety of other mitogenic stimuli may increase the binding of insulin to peripheral leucocytes. This may be particularly relevant in patients suffering from immunologically-mediated (e.g. autoimmune) diseases.

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Dr. F. Shimizu
Laboratory of Oral Medicine
National Institute of Dental Research
National Institutes of Health, Building 30, Room 121