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Cellular Responses Elicited by Insulin Mimickers in Cells Lacking Detectable Plasma Membrane Insulin Receptors

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Madin-Darby canine kidney (MDCK) cells were previously shown to have few or no plasma membrane insulin binding sites [Hofmann et al: J Biol Chem 258:11774, 1983]. Accordingly, neither insulin-stimulated incorporation of [^{14}C]glucose into glycogen, nor insulin-induced uptake of radiolabeled α-aminoisobutyrate ([^{3}H]AIB) could be demonstrated. To probe for receptors, MDCK cultures were surface-labeled with Na^{125}I or were labeled with [^{35}S]methionine. When solubilized cells were immunoprecipitated with sera containing antibodies to the insulin receptor, and immunoprecipitates were analyzed on SDS-gel electrophoresis, no evidence for insulin receptor components was found. Also, when intact MDCK cells were incubated first with serum containing antibodies to the insulin receptor and then with ^{125}I-protein A, no radiolabeling of insulin receptors occurred. Various agents reported to have insulin-like activity were tested on MDCK cells. The insulinomimetic lectins concanavalin A and wheat germ agglutinin as well as hydrogen peroxide enhanced incorporation of [^{14}C]glucose into glycogen and induced stimulated [^{3}H]AIB uptake, whereas trypsin, vanadate, and serum containing antibodies to the insulin receptor were without effects. Altogether, these results showed that MDCK cells had few or no insulin receptors and were correspondingly insulin-insensitive. However, since insulin-associated responses could be elicited by some insulin mimickers, the post-receptor limb of response in MDCK cells was apparently intact.

Key words: insulin receptors, insulin mimickers, insulin resistance

Insulin regulates multiple cellular responses ranging from acute effects on carbohydrate metabolism and transport of ions and amino acids to chronic effects on

Abbreviations used: MDCK, Madin-Darby canine kidney; H4, H4-II-EC3 hepatoma cells; AIB, aminoisobutyrate; con A, concanavalin A; WGA, wheat germ agglutin; SDS, sodium dodecyl sulfate; DTT, dithiothreitol; S.D., standard deviation.

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synthesis of protein and DNA. It is not known whether all these actions are mediated similarly, or whether each effect is evoked by a unique signal. The hormone binding site of the insulin receptor is generally thought to be the site at which the regulatory effects of insulin are initiated [1]. It is not clear whether metabolic and growth regulatory signals are generated from the receptor, are produced indirectly via a receptor-initiated signal cascade, or are alternatively generated at some point later in the course of cellular insulin/receptor processing. Insulin binding has been variously shown to stimulate phosphorylation of its receptor [2–4], to release plasma membrane-derived mediators that enhance or inhibit actions of intracellular enzymes [5–7], or to elicit nuclear effects indirectly or by direct interactions at nuclear membrane sites [8,9]. Studies examining the coupling of receptor binding and post-binding events associated with different cellular responses are necessary to clarify the physiologic actions of insulin.

In this study, we employed a deviant cell line as a tool to gain insight into normal signaling processes associated with cellular regulation by insulin. Madin-Darby canine kidney (MDCK) cells, derived from normal dog kidney, have been described as well-differentiated, with retention of kidney transport function [10,11] and responsiveness to glucagon via stimulated adenylate cyclase [12]. We reported earlier, however, that MDCK cells had few or no specific binding sites for radiolabeled insulin [13]; and in the present report, we examined whether insulin receptors could be detected using immunological methods. We found that MDCK cells were insulin-insensitive when evaluated for cellular activities normally enhanced by insulin, ie, glycogen synthesis and amino acid transport. We investigated further to probe the extent and nature of this cellular defect by examining whether responses could be elicited by known insulinomimetic agents including lectins, hydrogen peroxide, trypsin, vanadate, and serum containing antibodies to the insulin receptor. We suggest the potential of the MDCK cell line as a model for cellular insulin resistance owing to an insufficient number of plasma membrane insulin receptors.

MATERIALS AND METHODS

Chemicals

Radiochemicals and supplies including D-[U-^{14}C]glucose (230 mCi/mmol), NCS tissue solubilizer, and OCS counting fluid were obtained from Amersham Corp. (Arlington Heights, IL). [Methyl-^{3}H]aminoisobutyric acid (10 Ci/mmol) and Aquasol-2 were purchased from New England Nuclear (Boston, MA). Concanavalin A (type V), wheat germ agglutinin, mannose, N-acetylglucosamine, trypsin (type III), dithiothreitol, aprotinin, phenylmethylsulfonyl fluoride, and sodlium orthovanadate were purchased from Sigma Chemical Co. (St. Louis, MO). Monocomponent porcine insulin was kindly provided by Dr. William Bromer, Lilly Research Laboratories (Indianapolis, IN). Rabbit serum containing antibodies to the insulin receptor (A410) was provided by Dr. Steven Jacobs (Wellcome Research Laboratory, Research Triangle Park, NC). Lactoperoxidase was obtained from Boehringer-Mannheim (Indianapolis, IN) and wheat germ agglutinin bound to agarose from Vector Labs, Inc. (Burlingame, CA). Staphylococcal protein A (Pansorbin) was obtained from Calbiochem-Behring (San Diego, CA) and all reagents for gel electrophoresis were from Bio-Rad Laboratories (Richmond, CA).

Cell Culture

The continuously cultured MDCK and H4 cell lines were obtained and cultured as described previously [13,15].

Measurement of D-[U-^{14}C]Glucose Incorporation Into Glycogen

The assay for insulin or lectin-stimulated incorporation of [^{14}C]glucose into glycogen in monolayer cell cultures was performed as reported previously [15]. Cells were incubated in Swim's S-77 medium (KC Biologicals, Lenexa, KA) with 4 mM glucose and 1% bovine serum albumin for 18 hr prior to the assay. Incubation medium during the assay was Swim's S-77 at pH 7.4 containing 20 mM HEPES, 5 mM sodium bicarbonate, 1% bovine serum albumin, 1.0 mM D-glucose, and insulin, lectins, vanadate, or trypsin at indicated concentrations.

Measurement of [methyl-^{3}H]aminoisobutyrate Uptake

Confluent monolayer cultures of MDCK or H4 cells (1–3 $\times$ 10^6 cells/60 mm plate) were rinsed and incubated 10 min at 37°C with 1.45 ml Swim's S-77 medium containing 20 mM HEPES, 5 mM sodium bicarbonate, and 1% BSA. Insulin, lectins, trypsin, vanadate, or sera containing antibodies against the insulin receptor were added in 0.05 ml volume to final concentrations indicated, and cell cultures were incubated for 4 hr at 37°C. Cells were then rinsed and further incubated with 1.5 ml Swim's S-77 medium containing 0.75 μCi [^{3}H]aminoisobutyrate (AIB). After 20 min at 37°C, the incubation was terminated by medium aspiration, and rinsing of cells with cold, buffered saline. Cell monolayers were solubilized with 2 ml 0.5 M NaOH for 20 min, transferred to counting vials, and counted for radioactivity by liquid scintillation using 10 ml Aquasol-2 cocktail.

Immunodetection of Insulin Receptors in Whole Cells

Confluent monolayer cultures of H4 or MDCK cells (in 60-mm plates) were incubated for 120 min at 22°C in medium with normal rabbit antibodies (polyclonal serum A410) at indicated dilutions. Following this incubation, cells were rinsed and incubated and additional 60 min at 37°C in 1.5 ml medium with ^{125}I-protein A ($\sim$ 100,000 cpm). Cells were then rinsed, solubilized, and counted to determine cell-associated radioactivity.

Surface Iodination and Biosynthetic Labeling of Cellular Proteins

Intact cells were surface-labeled with Na^{125}I essentially as described elsewhere [16]. Briefly, confluent monolayer MDCK or Fao cell cultures (in 100-mm plastic dishes) were rinsed and incubated with intermittent stirring for 30 min at 22°C in phosphate-buffered saline containing 10 mM glucose, 2 U/ml lactoperoxidase, 1 U/ml glucose oxidase, and 0.3–0.5 mCi/ml Na^{125}I. After iodination, monolayers were washed extensively with phosphate-buffered saline. When proteins were biologically labeled using [^{35}S]methionine, confluent cultures were incubated with 0.1 mCi/ml [^{35}S]methionine in complete culture medium for 18 hr [17].

Cell Solubilization and Immunoprecipitation of the Receptor

Labeled cells were scraped from the plates with a rubber spatula and solubilized in 2 ml of 1% Triton X-100 in 50 mM HEPES buffer, pH 7.4, containing aprotinin

(1,000 trypsin inhibitor U/ml) and 2 mM phenylmethylsulfonyl fluoride. Insoluble aggregates were removed by centrifugation at 200,000g for 60 min at 4°C. When the cells were labeled with [^{35}S]methionine, insulin receptors were partially purified using a wheat germ agglutinin-agarose column.

Solubilized cells were incubated with human (B-2, B-6, B-9) or rabbit sera (A410) containing antibodies to the insulin receptor (1:100 dilution) or non-immune serum for 12–18 hr at 4°C [16]. Immunoprecipitation was accomplished by addition of a slight excess of protein A (Pansorbin) as previously described [16,17]. After 1 hr at 4°C, the suspension was centrifuged at 10,000g for 1 min. The pellets were washed twice with 1 ml 1.0% Triton X-100 and 0.1% SDS in 25 mM HEPES, pH 7.4, and once with 0.1% Triton X-100 in 25 mM HEPES, pH 7.4.

Electrophoresis

Immunoprecipitates were solublized by boiling for 3 min in 2% SDS, 0.1 M DTT, 10% glycerol, 0.01% bromophenol blue, 10 mM sodium phosphate, pH 7.0. Electrophoresis was performed according to Laemmli [18]. The acrylamide concentration of the resolving gel was 7.5%, while that of the stacking gel was 4%. The gels were stained with 0.25% Coomassie blue in 50% trichloroacetic acid, destained in 7% acetic acid (vol/vol), washed in 3% glycerol, dried, and autoradiographed with Kodak-X-Omat film. Molecular weights were calculated by using standard proteins as previously described [18].

RESULTS

In studies evaluating binding of ^{125}I-insulin to monolayer cultures, it was reported that MDCK cells possessed few or no surface insulin binding sites [13]. In contrast, insulin-sensitive hepatoma cells (H4) displayed numerous specific cell surface receptors; this was also the case for liver and adipocytes (Table I). The receptor-deficient MDCK cells were correspondingly insulin-insensitive when evaluated for cellular activities normally enhanced by insulin, i.e, glycogen synthesis and amino acid transport. Expected dose dependent responses to insulin were elicited in control H4 hepatoma cells (Fig. 1A,B).

Immunological detection of plasma membrane insulin receptors in intact cells was accomplished by incubation of monolayer cultures with rabbit serum containing antibodies to purified rat insulin receptors (serum A410) [19]. Following rinsing, cell-bound antibodies were detected by a secondary incubation with ^{125}I-labeled protein A. Binding of ^{125}I-protein A to MDCK cells previously treated with anti-insulin receptor antibodies failed to increase above background binding levels (Fig. 2A), indicating a lack of immunologically recognizable cell surface insulin receptors in this cell line. In contrast, the amount of cell-associated radioactivity on antibody-treated H4 cells was increased over background levels and was dependent upon the

TABLE I. Estimated Number of Insulin Receptors (Ro) From ^{125}I-Insulin Binding Studies

Cells	Ro/Cell	Reference
H4 hepatoma	30,000	Hofmann et al [15]
Adipocyte	50,000	Kahn [1]
Liver	100,000	Kahn [1]
MDCK	< 500	Hofmann, unpublished

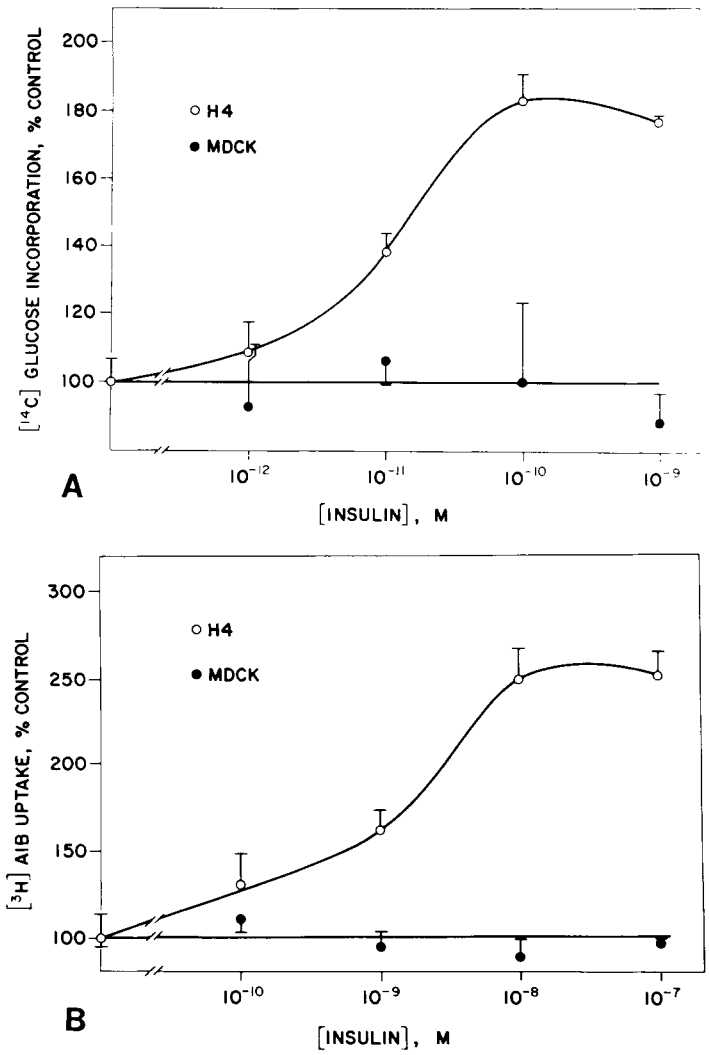

Fig. 1. A) Effect of insulin on incorporation of [^{14}C]glucose into glycogen in MDCK and H4 cells. MDCK and H4 monolayer cultures were incubated with indicated concentrations of insulin as described previously [13,15]. Cellular glycogen was then extracted, and radioactivity was determined. Data points are expressed as % basal incorporation and represent mean values ± S.D. for triplicate determinations. B) Effect of insulin on uptake of [^{3}H]AIB in MDCK and H4 cells. Confluent monolayer cultures of MDCK or H4 cells were incubated in medium containing indicated concentrations of insulin, and [^{3}H]AIB uptake was measured as described in Methods. Data points represent mean values ± S.D. for triplicate determinations.

dilution of immune serum in the initial incubation medium (Fig. 2B), thus confirming the presence of insulin receptors in H4 cells.

Further efforts to detect insulin receptors in MDCK cells were carried out using immunoprecipitation with various human sera containing specific antibodies to the receptor and using insulin-sensitive hepatoma cells as study controls. Confluent monolayers of cells were surfaced-labeled with Na^{125}I, cells were solubilized, and insulin receptors were immunoprecipitated and evaluated by SDS-polyacrylamide gel electrophoresis under reducing conditions. Electrophoresis of immunoprecipitates

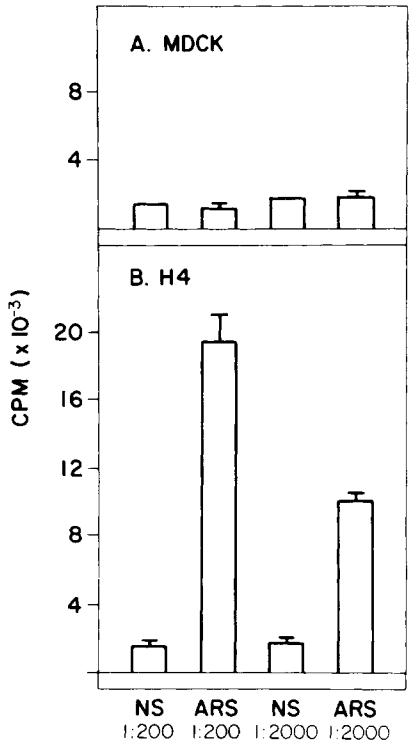

Fig. 2. Immunodetection of insulin receptors in intact MDCK (A) and H4 hepatoma (B) cultured cells. Confluent monolayer cultures of MDCK or H4 cells (in 60 mm plates) were incubated for 120 min at 22°C in 1.5 ml Swim's medium with normal rabbit serum (NS) or medium with rabbit serum containing anti-insulin receptor antibodies (ARS) at indicated dilutions. For detection of cell-associated immunoglobulins, cells were rinsed and incubated an additional 60 min at 30°C in 1.5 ml Swim's medium containing ^{125}I-protein A (~ 100,000 cpm). Cells were then rinsed, solubilized, and counted to determine cell-associated radioactivity. Data points represent mean value ± S.D. for triplicate determinations.

from hepatoma cells revealed the existence of two specific bands of M*r* 135,000 and 95,000 (Fig. 3) corresponding to the α and β subunits of the receptor reported in other studies [2]. In contrast, no specific bands were seen when MDCK cells were evaluated similarly (Fig. 3); the presence of non-specific bands was presumably due to overloading of the gels in an attempt to detect low amounts of labeled specific bands. To ascertain whether intracellular insulin receptors were present, cells were biologically labeled with [^{35}S]methionine, and receptors were partially purified on wheat germ agglutinin-agarose and immunoprecipitated using three human sera (B-2, B-6, and B-9) and a rabbit serum containing receptor antibodies. In MDCK cells, no insulin receptor components were detected in immunoprecipitates obtained with four different sera, while hepatoma cells exhibited characteristically labeled receptor subunits (data not shown).

To better understand the insulin insensitivity corresponding with the insulin binding defect in MDCK cells, metabolic effects of various known insulin-mimicking agents were also tested. The lectins Con A and WGA both elicited the insulin-associated effects of enhanced glycogen formation and stimulated induction of amino acid uptake. Con A maximally stimulated incorporation of [^{14}C]glucose into glycogen

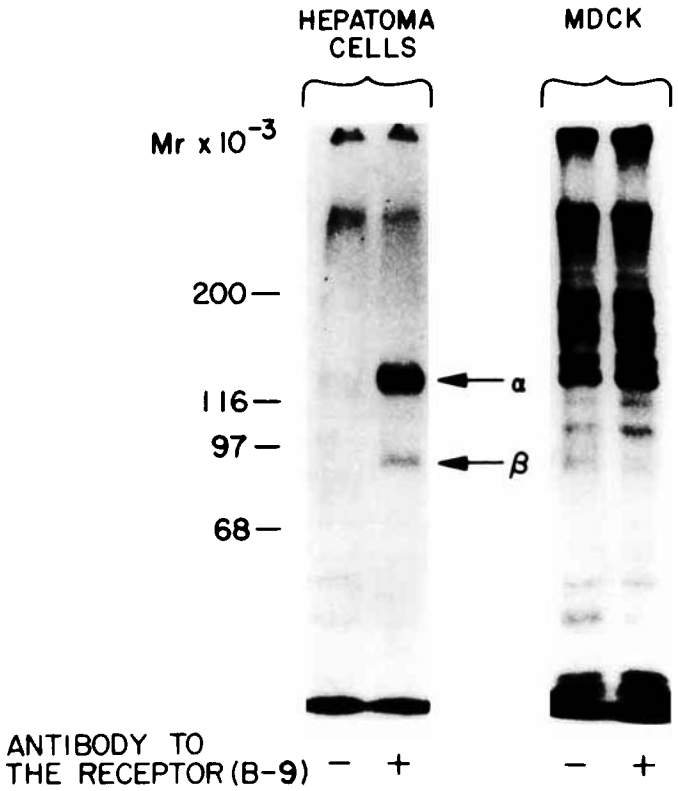

Fig. 3. Autoradiograms of gel electrophoresis of ^{125}I-labeled receptor immunoprecipitated by human anti-serum (B-9) in Fao hepatoma and MDCK cells. Intact monolayers of hepatoma cells (left) and MDCK cells (right) were surface-labeled with Na ^{125}I and lactoperoxidase, solubilized with 1% Triton X-100, and immunoprecipitated by pooled control or antireceptor B-9 sera. The immunoprecipitates were analyzed on SDS-polyacrylamide (7.5%) gel electrophoresis after reduction with 0.1 M dithiothreitol. B-9 serum antibodies to human insulin receptors recognized canine receptors since this serum (1:100 dilution) inhibited binding of ^{125}I-insulin to control canine liver membranes by >80%.

to >200% of basal value at concentrations of 30–60 μg/ml, while WGA in the same concentration range stimulated glycogen formation to >500% of the basal value (Fig. 4A). Induced [^{3}H]AIB uptake was maximally stimulated by WGA (280% of the basal value at 40 μg/ml lectin), and was also increased by Con A (140–150% of the basal value at 20–40 μg/ml) (Fig. 4B). Specificities of responses evoked by WGA and Con A were confirmed by the reversal of effects with inclusion of the respective lectin-binding monosaccharides N-acetylglucosamine and mannose in the incubation medium (results not shown).

Further experiments examined metabolic effects of the insulinomimetic agent hydrogen peroxide (H_2O_2) on MDCK cells. Such studies revealed dose-dependent enhancement of both [^{14}C]glycogen labeling and [^{3}H]AIB uptake induction by H_2O_2 in the concentration range .05–3 mM. Maximum stimulation of [^{14}C]glycogen formation to 252% of the basal value was elicited by 1.5 mM H_2O_2, while stimulation of [^{3}H]AIB uptake to 139% of the basal value was seen with 3.0 mM H_2O_2 (Fig. 5A,B).

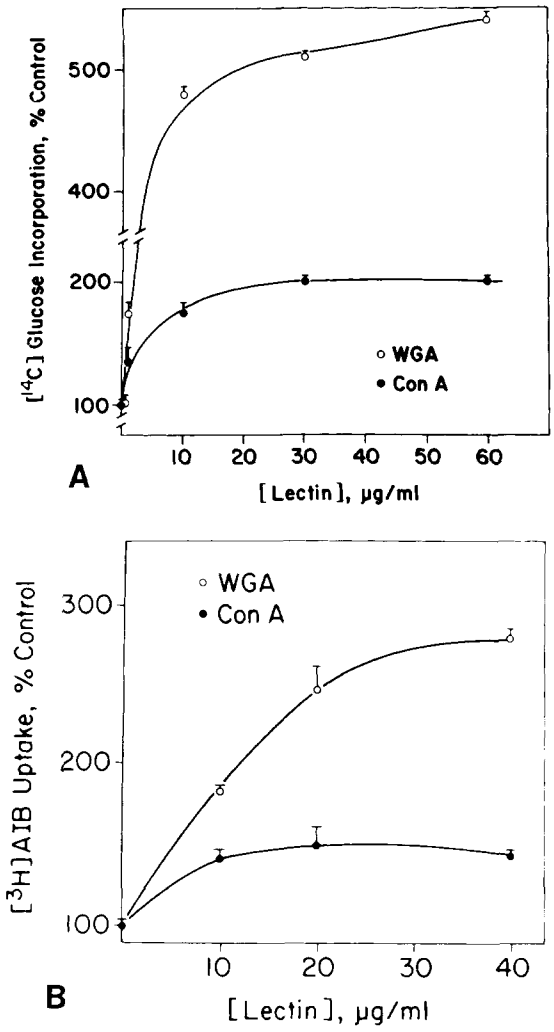

Fig. 4. A) Lectin stimulation of [^{14}C]glucose incorporation into glycogen by MDCK cells. Monolayer cultures of MDCK cells were incubated in medium containing indicated concentrations of Con A (-●-) or WGA (-○-). Incorporation of [^{14}C]glucose into glycogen was measured as described in Methods. Data points are expressed as % basal incorporation and represent mean value ± S.D. for triplicate determinations. B) Lectin stimulation of [^{3}H]AIB uptake by MDCK cells. Monolayer cultures of MDCK cells were incubated in medium containing indicated concentrations of Con A (-●-) or WGA (-○-). [^{3}H]AIB uptake was measured as described in Methods with data points representing mean values ± S.D. for triplicate determinations.

The insulinomimetic effect of serum containing antibodies against the insulin receptor was also tested. Monolayer cultures of MDCK and H4 cells were evaluated for the insulin-associated responses of stimulated [^{14}C]glycogen labeling and [^{3}H]AIB uptake using serum containing anti-receptor antibodies and normal rabbit serum (1:200 and 1:2,000, serum dilutions). MDCK cells did not respond to anti-receptor serum in ether biologic assay, whereas H4 hepatoma cells responded to anti-receptor

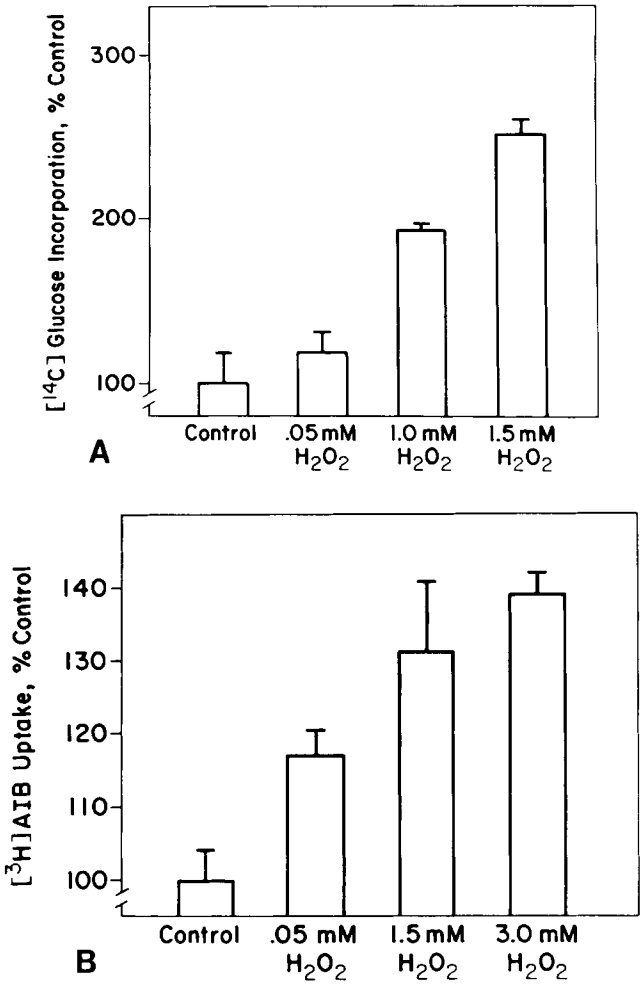

Fig. 5. A) Hydrogen peroxide stimulation of [^{14}C]glucose incorporation into glycogen by MDCK cells. Confluent monolayer MDCK cell cultures were incubated in medium containing hydrogen peroxide (H_2O_2) at indicated concentrations. Formation of [^{14}C]glycogen was measured as described in Methods. Data points are expressed as % basal incorporation and represent mean values ± S.D. for triplicate determinations. B) Hydrogen peroxide stimulation of [^{3}H]AIB uptake by MDCK cells. MDCK cell cultures were incubated in medium containing H_2O_2 at indicated concentrations. [^{3}H]AIB uptake was measured as described in Methods. Data points are expressed as % basal incorporation and represent mean values ± S.D. for triplicate determinations.

with 29% and 57% increased [^{14}C]glycogen formation and 34% and 54% stimulated [^{3}H]AIB uptake at the high and low serum dilutions, respectively. Neither cell line showed any response to normal rabbit serum (Table II).

The insulin-mimicking protease trypsin (at .01–2.0 μg/ml) stimulated [^{14}C]glucose incorporation into glycogen in H4 cells up to 147% of the basal value, but failed to show a like effect in MDCK cells (Table III). Similarly, trypsin (1.0 μg/ml) induced stimulated [^{3}H]AIB uptake to 152% of basal value in H4 cells and showed

TABLE II. Effect of Anti-Receptor Antibodies on Glycogen Formation and Amino Acid Uptake in H4 and MDCK Cells

Agent, dose	H4 cells	% Basal	MDCK cells	% Basal
Exp. 1	[^{14}C]Glycogen, cpm		[^{14}C]Glycogen, cpm	
None	2,327 ± 267[a]	100	29,061 ± 967	100
ARS, 1:200[b]	3,646 ± 193	157	26,995 ± 2,674	93
1:2000	3,003 ± 53	129	29,918 ± 2,057	102
NRS, 1:200[b]	1,825 ± 118	78	27,283 ± 1,413	94
1:2,000	1,918 ± 224	82	27,206 ± 2,999	94
Exp. 2	[^{3}H]AIB uptake, cpm		[^{3}H]AIB uptake, cpm	
None	8,349 ± 546	100	6,794 ± 290	100
ARS, 1:200	12,882 ± 758	154	6,322 ± 118	93
1:2,000	11,148 ± 716	134	6,637 ± 275	98
NRS, 1:200	8,825 ± 30	106	5,894 ± 365	87
1:2,000	8,762 ± 842	105	6,962 ± 94	102

[a]Mean value ± S.D. for triplicate determinations.
[b]NRS is normal rabbit serum, ARS is anti-insulin receptor serum at indicated dilutions.

a slight, but not significant, increase over basal levels in MDCK cells (Table III). Another mimicker, sodium orthovanadate (0.1–10 μM) was effective in stimulating [^{14}C]glycogen labeling in H4 cells but was without effect in MDCK cells (Table IV). Stimulated uptake of aminoisobutyrate was not elicited by vandate in either cell line (data not shown).

DISCUSSION

Decreased cellular sensitivity to the action of insulin is a condition associated with obesity, type II diabetes, and types A and B insulin resistance syndromes. Such insulin resistance represents a condition in which a given concentration of insulin produces less than the expected biologic effect, and three general causes have been identified: (a) abnormalities of the insulin molecule, (b) circulating antagonists of insulin action, and (c) target cell defects in the pathways for insulin action [20]. At the target cell level, post-receptor defects may play a role in insulin resistance, although in obese patients or patients with impaired glucose tolerance and mild insulin resistance, diminished insulin sensitivity can be attributed primarily to a decreased number of insulin receptors [20].

MDCK cells were exploited as an in vitro model for cellular insulin resistance. We previously reported that specific membrane binding of radiolabeled insulin to MDCK cells was undetectable [13]. Presently we report that with immunodetection techniques using human or animal sera containing insulin receptor-specific antibodies, neither intact insulin receptors nor α and β subunits could be demonstrated in MDCK cells. We also found that insulin failed to enhance glycogen formation or induce stimulated uptake of the alanine analogue AIB in MDCK cells. Thus, MDCK cells appeared to have few or no specific insulin receptors and were likewise metabolically insensitive to insulin. To ascertain the nature and extent of the MDCK cellular resistance to insulin, biologic effects were examined for known insulin-mimickers—the lectins Con A and WGA, trypsin, vanadate, hydrogen peroxide, and serum containing antibodies to the insulin receptor. While all agents tested displayed insulin-

TABLE III. Effect of Trypsin on Glycogen Formation and Amino Acid Uptake in H4 and MDCK Cells

Agent, dose	H4 cells	% Basal	MDCK cells	% Basal
Exp. 1	[^{14}C]Glycogen, cpm		[^{14}C]Glycogen, cpm	
None	4,542 ± 461[a]	100	29,276 ± 1,907	100
Trypsin, 0.01 μg/ml	4,939 ± 271	109	26,780 ± 1,655	91
1.0 μg/ml	5,657 ± 507	125	18,167 ± 1,291	62
2.0 μg/ml	6,698 ± 116	147	15,229 ± 2,376	52
Insulin, 6 ng/ml	8,919 ± 1116	196	—	—
WGA, 30 μg/ml	—	—	80,707 ± 6,437	230
Exp. 2	[^{3}H]AIB uptake, cpm		[^{3}H]AIB uptake, cpm	
None	3,031 ± 23	100	2,862 ± 250	100
Trypsin, 0.1 μm/ml	3,330 ± 84	110	3,105 ± 331	109
1.0 μg/ml	4,621 ± 302	152	3,291 ± 431	115
Insulin, 6 ng/ml	5,733 ± 252	189	—	—
WGA, 30 μg/ml	—	—	6,134 ± 259	214

[a]Mean value ± S.D. for triplicate determinations.

TABLE IV. Effect of Vanadate on Glycogen Formation in H4 and MDCK Cells

Agent, dose	H4 cells	% Basal	MDCK cells	% Basal
Exp. 1	[^{14}C]glycogen, cpm		[^{14}C]glycogen, cpm	
None	2,917 ± 113[a]	100	34,887 ± 1,816	100
Vanadate, 0.1 μM	3,756 ± 311	128	34,454 ± 2,624	99
1.0 μM	4,146 ± 216	142	35,760 ± 1,928	103
10.0 μM	5,882 ± 525	202	34,958 ± 2,575	100
Insulin, 10^{-10} M	8,950 ± 193	307	—	—

[a]Mean value ± S.D. for triplicate determinations.

like activities in the insulin-sensitive H4 hepatoma cells, only the lectins and hydrogen peroxide acted to stimulate glycogen synthesis and amino acid uptake in MDCK cells (Table V). Such findings indicated that it was possible in MDCK cells to bypass plasma membrane insulin binding sites but still elicit cellular responses characteristically mediated by insulin.

While binding of insulin to its plasma membrane receptor is generally acknowledged as central to the actions of insulin, there have been experimental conditions reported whereby the hormone binding site can apparently be bypassed, but intact post-binding response pathways can be demonstrated. Pillion and coworkers [21,22] showed that an insulinomimetic antibody activated glucose transport in adipocytes via interaction with a membrane protein that was neither the insulin receptor nor the glucose transporter. Also, Shechter and Sela [23] demonstrated that the lectin wax bean agglutinin maintained its ability to elicit insulin-like responses in adipocytes with markedly reduced insulin binding owing to prior cell treatment with trypsin. Further, Shechter [24] reported that persistent insulinomimetic activity resulted from WGA permanently adsorbed to adipocyte cell surface determinants and that adsorbed lectin was not internalized or processed as is receptor-bound insulin. Each of these insulin-associated effects was considered compatible with a concept of direct interaction of

TABLE V. Summary: Ability of Insulin-Mimicking Agents To Stimulate Glycogen Formation and Amino Acid Uptake in H4 and MDCK Cells

	[^{3}H]AIB uptake[a]		[^{14}C]Glucose incorporation into glycogen[a]	
Agent	H4	MDCK	H4	MDCK
Wheat germ agglutinin	++	+++	++	++++
Con A	+	++	++	+++
Hydrogen peroxide	+	+	+	++++
Trypsin	+	−	+	−
Vanadate	−	−	++	−
Anti-receptor antibody	++	−	++	−

[a]Stimulation represented as percent basal activity as follows: <120% = −; 120–150 = +; 150–200 = ++; >200 = +++; >300 = ++++.

ligand with plasma membrane "effector" or "signal transducer" distinct from plasma membrane insulin binding sites.

Alternatively, the insulin-like effects of lectins on MDCK cells might also be due to cellular activation via plasma membrane receptors for other hormones. Evidence is rapidly accumulating for coordinated control of cellular function by various peptide hormones including insulin, insulin-like growth factors (IGF) I and II, epidermal growth factor, and glucagon, by mechanisms of receptor cross-reactivity for hormone binding [25], cross-regulation of receptor binding properties [26], and co-regulatory or counter-regulatory control of enzyme systems [27]. That insulin failed to stimulate glycogen synthesis and AIB transport even though insulin reportedly has a low affinity for the IGF-I receptor [25] is inconsistent with this hypothesis.

Another possible mechanism for insulin-like effects of lectins even in the apparent absence of specific plasma membrane insulin binding sites is that lectins may exert direct metabolic effects following delivery by endocytosis to intracellular regulatory sites. Purello et al have demonstrated direct effects of insulin, the lectins con A and phytohemagglutinin, and antiserum to the insulin receptor on nuclear envelope phosphorylation [8]. Shechter, however, has reported that plasma membrane-bound WGA was not internalized but was nevertheless lipogenic in adipocytes [24]. Further studies will be required to understand insulinomimetic actions of lectins.

Antibodies directed against the insulin receptor have been shown to mimic an extensive array of acute metabolic effects of insulin [14] and have recently been reported also to simulate the developmental role of insulin [28]. Since the polyclonal serum containing anti-insulin receptor antibodies used in these studies was capable of immunoprecipitating receptor components [19], an antibody-receptor interaction would presumably be the initial step for response activation. In addition, vanadate was shown to elicit such insulin-like metabolic effects as enhancement of glucose oxidation [29], activation of glycogen synthase [30], and inhibition of lipolysis [29]. As vanadate stimulated both receptor phosphorylation and glycogen synthase, it was speculated that these may be related actions [30]. Similarly, the protease trypsin stimulated phosphorylation of the insulin receptor and also activated rat adipocyte glycogen synthase [31]. The failure of anti-insulin receptor antibody, vanadate, and trypsin to elicit insulin-like effects in the insulin-receptor-deficient MDCK cells

supports the contention that these agents normally function via receptor-initiated mechanisms.

Hydrogen peroxide was shown by Czech et al [32] to mimic insulin by activating white fat cell glucose oxidation. This agent presumably acted at a step beyond the cell surface site that bound the hormone, since trypsinization of fat cells destroyed the capacity of the insulin receptor to bind insulin [33,34] without altering the fat cell response to hydrogen peroxide [35]. Also, Knight et al reported that leprechaun patient fibroblasts with defective insulin receptors had a markedly decreased ability to respond to insulin but responded normally to hydrogen peroxide with stimulated [^{14}C]glucose incorporation [36]. Stimulation of glycogen synthesis and amino acid uptake in insulin-resistant MDCK cells by hydrogen peroxide was consistent with its initial site of action beyond the plasma membrane insulin binding site.

In summary, our experimental studies in receptor-deficient MDCK cells showed that known insulinomimetic agents, ie, lectins and hydrogen peroxide, could elicit insulin-associated effects of stimulated amino acid uptake and enhanced glycogen formation. These findings indicated that the impaired response to insulin in MDCK cells could be attributed to an insufficient number of specific insulin binding sites rather than a defect in the post-binding limb of response. Following insulin binding to its specific plasma membrane receptor, the precise signal by which insulin regulates intracellular responses remains to be identified. Likewise, alternative points of entry into the hormone action sequence must be identified for insulinomimetic agents such as lectins and hydrogen peroxide. Further studies with MDCK cells may help increase our understanding of the mechanism coupling insulin receptor binding and post-binding responses.

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