Inhibition of Endosomal Acidification in Normal Cells Mimics the Derangements of Cellular Insulin and Insulin-Receptor Metabolism Observed in Non–Insulin-Dependent Diabetes Mellitus

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Dissociation of the insulin–insulin receptor complex plays a crucial role in the processing of both insulin and the insulin receptor, and the acidification of endocytic vesicles may be the mechanism by which internalized insulin is dissociated from its receptor and properly sorted and processed. Internalized insulin–insulin receptor complexes are abnormally processed in cells from patients with non–insulin-dependent diabetes mellitus (NIDDM). Accordingly, to further investigate the mechanisms of the derangements observed in NIDDM cells, we examined the effects of the ionophore monensin, which inhibits endosomal acidification, on the cellular processing of insulin and insulin receptor in monocytes from control subjects (n = 12) and NIDDM patients (n = 14). This study confirms that monocytes from NIDDM patients, compared with cells from normal controls, had reduced binding ($P < .01$), internalization ($P < .01$), and degradation ($P < .01$) of insulin. In addition, the release of intracellular radioactivity was slower ($P < .01$), and recycling of the insulin receptor was inhibited ($P < .01$). Moreover, these defects were associated with a significant ($P < .01$) decrease of dissociation of the internalized insulin–insulin receptor complex. In cells from normal controls, incubation with monensin decreased insulin binding ($P < .01$), but not insulin internalization. High-performance liquid chromatography (HPLC) analysis of intracellular radioactivity showed that after monensin intracellular intact insulin significantly increased ($P < .01$), thus suggesting a decrease of intracellular insulin degradation. Moreover, insulin receptor recycling was completely disrupted. All of these derangements were associated with a significant decrease ($P < .01$) of dissociation of insulin–insulin receptor complexes. On the contrary, in diabetic monocytes, monensin had no significant additional effect on NIDDM-linked alterations. Comparison of the results obtained in cells from NIDDM patients to those found in monensin-treated normal cells demonstrates that NIDDM and monensin gave rise to a superimposable impairment of dissociation of the intracellular insulin–insulin receptor complex, associated with similar abnormal sorting and processing of insulin and its receptor. The only defect present in NIDDM cells but not in monensin-treated cells is the decrease of insulin internalization, which thus seems independent of the action of monensin on the processing of internalized insulin–insulin receptor complex. These results suggest that the impairment of dissociation of the insulin–insulin receptor complex may play a crucial role in the subsequent altered processing of insulin and insulin receptor. Moreover, they raise the question as to a possible similar alteration of the same intracellular mechanism by NIDDM and monensin, and point out that the derangements found in cells from NIDDM patients could be localized within the endosomal apparatus and consist mainly of a defective acidification of its interior.

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AFTER INSULIN BINDS to its receptor, the insulin–insulin receptor complex enters the internal milieu of the cell by the endosomal apparatus. The sorting of internalized insulin from its receptor consists of two sequential steps: dissociation followed by segregation into separate compartments, which allows insulin to be degraded and receptor recycled back to the plasma membrane. Dissociation of the insulin–insulin receptor complex is accomplished by the acidic pH of endosomes obtained by the action of an ATP-dependent proton pump.[1,2]

Cells from patients with non–insulin-dependent diabetes mellitus (NIDDM) have multiple postbinding defects of insulin and insulin receptor intracellular trafficking and processing, including a decreased ability to internalize receptor-bound insulin, to degrade internalized insulin,[3-7] and to recycle back to the plasma membrane the insulin receptor.[6,7] Moreover, cells from insulin-resistant subjects show a defect of intracellular dissociation of the insulin-receptor complex[7] that could be responsible for alterations of both receptor recycling and insulin processing.

To better characterize the molecular mechanisms that underlie the defects observed in NIDDM, we inhibited endosomal acidification by monensin[8] to perturb insulin and insulin receptor processing in monocytes from normal controls and NIDDM patients. Moreover, we compared the effects of monensin in normal cells with those naturally associated with NIDDM.

SUBJECTS AND METHODS

Twelve normal subjects and 14 non-obese patients with NIDDM were studied after provision of informed consent for participation in the study, which was approved by the University of Pisa Medical School Ethics Committee.

Characteristics of the subjects are shown in Table 1 and indicate that the two groups were closely matched for age and body weight, whereas fasting plasma glucose and insulin were significantly higher in NIDDM patients.

All diabetic patients were diagnosed according to National Diabetes Data Group criteria.[9] They had a 3- to 6-month history of hyperglycemia. However, none had been treated with either insulin or oral antidiabetic agents. After they had fasted for 12 to 14 hours, blood

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Table 1. Study Group Characteristics

Group	Age (yr)	Body Mass Index (kg/m²)	Plasma Glucose (mmol/L)	Plasma Insulin (pmol/L)	Hemoglobin A_{1c} (%)
Control subjects (n = 12)	57.1 ± 8.9	24.7 ± 2.4	4.7 ± 0.6	43.2 ± 18	4.6 ± 0.5
NIDDM patients (n = 14)	59.0 ± 5.1	25.0 ± 3.4	8.2 ± 1.8*	75.0 ± 24*	7.9 ± 1.3*

*$P < .01$ *v* control subjects.

samples (150 mL) for cellular studies were drawn into a 3.8% citrate solution at 8 to 9 AM.

Highly purified human insulin (Novo, Copenhagen, Denmark) was iodinated by the lactoperoxidase method, and (A_{14}-^{125}I)-insulin (A_{14}-insulin) was subsequently separated from the iodination mixture using reverse-phase high-performance liquid chromatography (HPLC).[10] The specific activity of the tracer was 340 to 360 μCi/μg.

Mononuclear leukocytes were prepared according to the method of Boyum.[11] The percentage of monocytes determined using the latex bead method was 13% to 22% of the total cells. To study total cell-associated and internalized radioactivity, cells (33×10^6/mL) were incubated in 50 mmol/L HEPES, 120 mmol/L NaCl, 1.2 mmol/L $MgSO_4$, 5.0 mmol/L KCl, 15 mmol/L sodium acetate, 10 mmol/L glucose, 1 mmol/L EDTA, and 1% bovine serum albumin (BSA), pH 7.8, at 37°C for 60 minutes with 8.33 nmol/L A_{14}-insulin in the absence or presence of 16.6 μmol/L unlabeled insulin. Cell aliquots were then centrifuged in a Beckman microfuge (Palo Alto, CA) for 2 minutes at $8,000 \times g$, the supernatant was discarded, and the pellet radioactivity was counted in a gamma counter (Packard, Downers Grove, IL). To indicate the radioactivity in the cell pellets, the term total cell-associated radioactivity is used. To determine the percentage of radioactivity that was internalized, the pellets were washed once with 500 μL chilled binding buffer and then acid-washed.[3] In control experiments, we have observed that the acid wash procedure removes more than 98% of insulin bound at 4°C to the membrane receptor of monocytes. Moreover, the results of acid washing in monocytes incubated with insulin at 37°C are superimposable on those obtained with trypsin treatment.[12] After the acid wash, cell viability remained greater than 90% as assessed by trypan blue exclusion.

Radioactivity that was not extractable with the acid wash procedure was considered internalized. To determine the nature of the internalized radioactivity, monocytes were solubilized in 1 mL 30-mmol/L phosphate-buffered saline (PBS) containing 4 mol/L urea, 1 mol/L acetic acid, and 0.1% Triton X-100, pH 2.5, at 4°C for 1 hour. The solubilized material was centrifuged at $8,000 \times g$ (2 minutes at 4°C), and the supernatants were analyzed by HPLC. Chemicals were obtained from Merck (Darmstadt, Germany). Deionized distilled water purified with a Milli-Q System (Millipore, Bedford, MA) was used for reagent preparation. Before use, all eluents were degassed under vacuum. A preliminary step to HPLC procedures was the extraction of radioactivity from solubilized monocytes using a Sep-Pak C18 cartridge (Millipore)[13] activated by elution in sequence 5 mL methanol and 10 mL H_2O, and the residuate water was purged by passing 20 to 30 mL of a nitrogen flow. After loading the sample, the Sep-Pak cartridge was washed with 10 mL H_2O, thus washing away unbound components. Elution of bound peptides from the cartridge was then performed with 5 mL of a phase containing acetonitrile and phosphate buffer 0.01 mol/L (80:20 vol/vol), pH 3. Eluent was collected in 1-mL fractions by applying vacuum to the column. Recovery of radioactivity from the Sep-Pak C18 column was always greater than 98%. The isocratic HPLC system was obtained from Waters Associates (Milford, MA) and consisted of a model 510 pump, a model U6K injector with a 2-mL loop, and a model 441 UV absorbance detector. HPLC analysis was performed using a Waters C18 μBondapak (average particle size, 10 μm) column (300×3.9 mm ID), with a mobile phase of 0.01 mol/L sodium phosphate buffer:isopropanol: acetonitrile (68:11:21 vol/vol/vol) containing 0.15 mol/L ammonium acetate (11.56 g/L eluent) and adjusted to pH 3 with hydrochloric acid.[13]

Additional studies were performed to measure the rate of release of radioactivity from monocytes to an insulin-free medium after insulin internalization and intracellular degradation. Cells were incubated with 8.33 nmol/L labeled insulin in HEPES buffer for 60 minutes at 37°C, acid-washed to remove insulin bound to surface receptors, and then resuspended in insulin-free buffer at 37°C. The amount of intracellular radioactivity that remained cell-associated was evaluated at different times by centrifuging cell aliquots in a Beckman microfuge and then washing and counting the cell pellets. Cell viability measured at the end of the studies by the trypan blue exclusion technique was always greater than 90%.

Insulin receptor internalization and recycling was evaluated by incubating monocytes with 100 nmol/L unlabeled insulin in binding buffer, pH 7.8, for 60 minutes at 37°C to obtain maximal receptor internalization.[6] After acid washing to remove insulin bound to surface receptors, cells were incubated at 37°C for different times to allow internalized receptor to return to the cell surface. Insulin binding was measured before and after cell exposure to unlabeled insulin and at 15 minutes, 30 minutes, and 45 minutes during rewarming. To perform insulin binding, cells were incubated in duplicate in 50 mmol/L HEPES buffer, 1% BSA, pH 7.8, at 12°C for 180 minutes with A_{14}-insulin (34 pmol/L). Aliquots of the suspension were added to a 1.5-mL microtube containing 200 μL HEPES. Cell-bound and free insulin was separated by centrifugation in a Beckman microfuge for 1 minute. Bound A_{14}-insulin was determined by a gamma counter. Nonspecific binding was determined in the presence of 16.6 μmol/L unlabeled insulin. The amount of internalized A_{14}-insulin bound to insulin receptor was measured using a polyethylene glycol (PEG) precipitation assay,[14] modified from the procedure originally described for the insulin receptor.[15] Following a 60-minute incubation with labeled insulin at 37°C, cells were washed three times with ice-cold PBS and then solubilized in ice-cold 0.5% Triton X-100 (which does not disrupt the insulin-receptor complex[16]) in 25 mmol/L HEPES, 150 mmol/L NaCl, and 0.1 mg/mL aprotinin, pH 7.4. Cells were solubilized for 5 minutes with gentle swirling. Then, two aliquots were removed. One was counted directly to determine total cell-associated radioactivity; to the other were added 350 μL 1% γ-globulin in PBS and 700 μL 25% PEG. The samples were centrifuged in a Beckman microfuge at 4°C for 5 minutes. The supernatant was removed, and radioactivity in the pellet was counted. Under these experimental conditions, less than 2% of standard A_{14}-insulin was precipitated. This value was subtracted from the value obtained when solubilized radioactivity was tested.

All cellular studies either with cells from normal subjects or with cells from NIDDM patients were performed in the absence or presence of monensin (Sigma, St Louis, MO). Aliquots of cells were preincubated with 50 μmol/L monensin for 60 minutes at 37°C in binding buffer. After this time, labeled insulin was added and the studies were performed. In preliminary experiments, 50 μmol/L monensin had proven able to decrease intracellular insulin degradation in normal cells to a value superimposable on that observed in cells from NIDDM patients. When monocytes were incubated with labeled insulin in the continuous presence of monensin, total cell-associated and internalized radioactivity reached a steady state after 60 minutes, a value superimposable on that obtained in the absence of monensin (data not shown). To evaluate cell-associated monensin, 1 mL solubilized cell sample was mixed with 3 mL ethylacetate:butanol (9:1 vol/vol) and centrifuged at $15,000 \times g$ for 20 minutes. The supernatant was lyophilized, resuspended in methanol 200 μL, and analyzed by HPLC using a C18 μBondapack column with a mobile phase of sodium phosphate buffer: acetonitrile (70.8:29.2 vol/vol), pH 7.0. Monensin was detected by UV absorption at 204 nm. The linearity of the method was tested by plotting

the HPLC peak heights of monensin against its concentrations in standard solutions. The plots were linear ($r = .99$) and passed through the origin.

All results of the studies are expressed as the mean ± SD. Statistical significance was assessed using two-way ANOVA and two-tailed Student's t test for paired and unpaired data.

RESULTS

Basal studies performed at 37°C confirmed the reduced amount of total cell-associated radioactivity in cells from NIDDM patients (2.63% ± 0.7%, $P < .01$) compared with cells from control subjects (4.7% ± 0.8%). Furthermore, the percentage of total cell-associated radioactivity that was internalized was significantly decreased in monocytes from diabetics (33.2% ± 6%, $P < .01$) compared with control subjects (49.6% ± 9%). The presence of monensin in the incubation medium of control cells induced a decrease of total cell-associated radioactivity (3.7% ± 0.7% *v* 4.7% ± 0.8%, $P < .01$). On the contrary, no effect of monensin was observed in the percentage of cell-associated radioactivity that was internalized. Monensin had no effect in cells from NIDDM patients either on total cell-associated radioactivity or internalized radioactivity (Fig 1).

The characterization of intracellular radioactivity by HPLC showed, as previously reported, that intracellular undegraded insulin was present at a higher percentage in cells from NIDDM patients (26.2% ± 7.2%, $P < .01$) than in those from control subjects (5.1% ± 2%). Nevertheless, in the presence of monensin, the percentage of intracellular intact insulin within normal cells significantly increased (26.4% ± 9% *v* 5.1% ± 2%, $P < .01$), thus confirming that monensin inhibits intracellular insulin degradation. When the effect of monensin was evaluated in cells from NIDDM patients, no significant modification of intracellular intact insulin was observed (32.1% ± 10% *v* 26.2% ± 7.2%) (Fig 2).

In the absence of monensin, the loss of internalized radioactivity from the intracellular compartment was significantly higher (ANOVA, $P < .05$) in normal cells than in diabetic cells. When normal cells were studied in the presence of monensin, the loss of intracellular radioactivity was decreased (ANOVA, $P < .05$) to the value observed in NIDDM cells in which no effect of monensin was detectable (Fig 3).

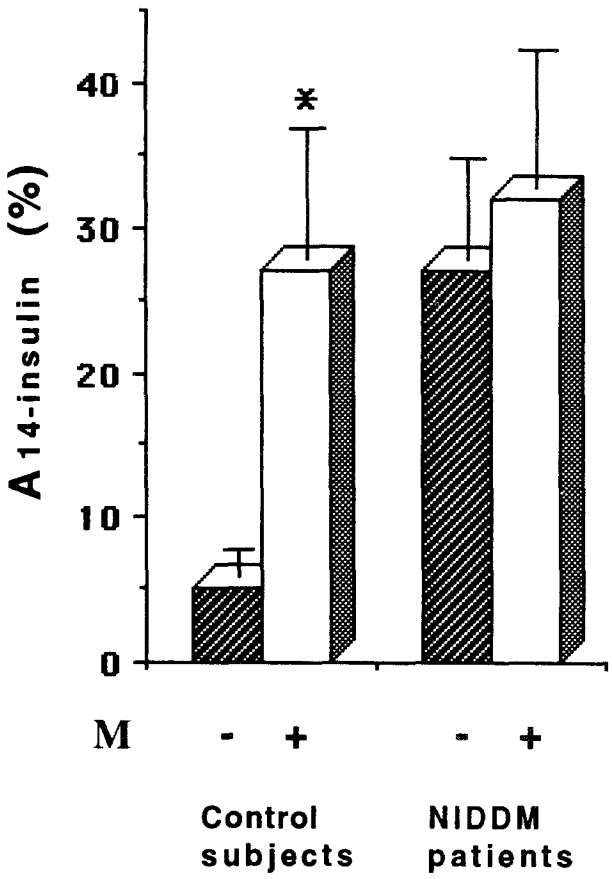

Fig 2. Intracellular intact insulin expressed as a percent of intracellular radioactivity in monocytes from control subjects and NIDDM patients. After incubation with A_{14}-insulin for 60 minutes at 37°C in the continuous presence (+) or absence (−) of monensin (M), cells were acid-washed and solubilized. Intracellular intact insulin was determined by HPLC. *$P < .01$ *v* control M−.

To study receptor internalization and recycling, cells were first incubated with unlabeled insulin for 60 minutes at 37°C,

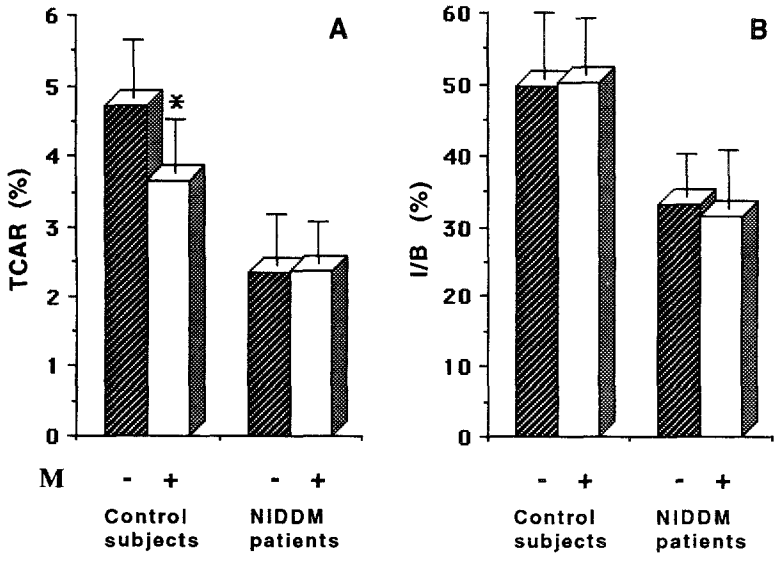

Fig 1. (A) Total cell-associated radioactivity expressed as a percent of radioactivity added to the incubation medium (TCAR) and (B) internalized radioactivity expressed as a percent of TCAR (I/B) in monocytes from control subjects and NIDDM patients in the continuous presence (+) or absence (−) of monensin (M). *$P < .01$ *v* control M−.

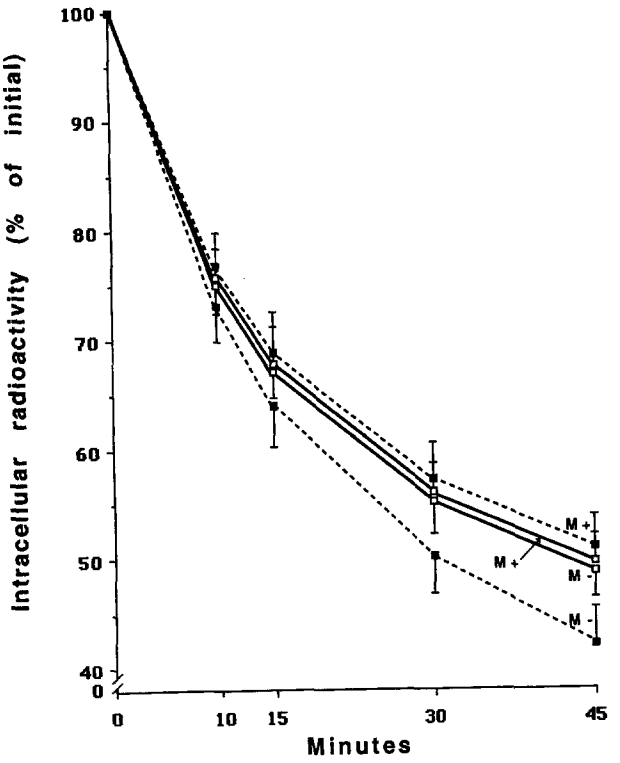

Fig 3. Loss of intracellular radioactivity (% of initial) in monocytes from control subjects (■) and NIDDM patients (□) in the continuous presence (+) or absence (−) of monensin (M). Cells were incubated at 37°C for 60 minutes with A_{14}-insulin, acid-washed, and then resuspended at 37°C in insulin-free medium. The loss of intracellular radioactivity in control M+ is significantly different (ANOVA, $P < .05$) compared with that of control M−.

acid-washed, and rewarmed in insulin-free binding buffer to allow recovery of cell-surface insulin binding. In down regulated normal cells, the percent decline of insulin binding was significantly higher (42% ± 18%, $P < .01$) than in diabetic cells (24% ± 13%). Moreover, normal cells almost completely recovered the basal insulin binding after rewarming, whereas diabetic cells had little or no recovery. These results confirm those of a previous study[6] and suggest a reduced insulin receptor internalization and recycling in NIDDM patients. Incubation of normal cells with monensin slightly reduced the initial insulin binding but not the percent decrease of insulin binding after cell exposure to insulin (44% ± 16%), whereas insulin receptor recycling was inhibited. In diabetic cells, monensin showed no effect on initial and residual insulin binding or on receptor recycling (Fig 4).

Intracellular insulin–insulin receptor complexes were determined from the PEG-precipitable radioactivity measurement after cell incubation for 60 minutes at 37°C with labeled insulin, and thereafter at different times during reincubation of cells at 37°C in an insulin-free buffer. These experimental conditions were those used to evaluate insulin receptor recycling and exocytosis of intracellular radioactivity. The percentage of PEG-precipitable radioactivity was significantly lower in normal controls than in NIDDM patients (7.5% ± 4% *v* 17.5% ± 6%, $P < .01$; Fig 5A). Moreover, the time course of intracellular PEG-precipitable material showed a greater decline in normal controls than in diabetic patients (ANOVA, $P < .01$; Fig 5B). After monensin, PEG-precipitable material in normal cells increased (20% ± 6%, $P < .01$), but no effect was observed in diabetic monocytes (19.2% ± 4%; Fig 5A). Monensin also significantly reduced (ANOVA, $P < .05$) the time course of PEG-precipitable material in normal cells, but exerted only a marginal effect in diabetic cells (Fig 5B).

Monensin is a highly lipophilic compound that presumably can be inserted into the cellular membrane and eventually internalize. We evaluated by HPLC the amount of cell-associated monensin (Fig 6). The results obtained show that monensin was associated at superimposable concentrations with cells from normal controls (21.8 ± 1 μmol/L) and NIDDM patients (19.9 ± 1 μmol/L).

DISCUSSION

This study confirms that monocytes from NIDDM patients have reduced binding, internalization, and degradation of insulin and inhibited recycling of the insulin receptor. Moreover, it shows that these defects are associated with a decrease of dissociation of the insulin-receptor complex. Our results have been obtained with freshly isolated monocytes that can be influenced by derangements of the diabetic milieu such as hyperinsulinemia and/or hyperglycemia. Nevertheless, cultured Epstein-Barr virus–transformed lymphocytes from insulin-resistant subjects have shown a delayed dissociation of the insulin-receptor complex associated with an impairment of both recycling of the receptor to the cell surface and degradation of internalized insulin.[7] This latter result provides the evidence that a primary defect of intracellular insulin and insulin receptor processing is present in cells from insulin-resistant patients. Moreover, taken together, these data suggest the existence of a tight link between the impairment of dissociation of the insulin-receptor complex and the derangements of intracellular insulin and insulin receptor processing. Dissociation of the internalized insulin–insulin receptor complex may play a crucial role in insulin and insulin receptor processing,[2] and the acidification of endocytic vesicles may be the mechanism by which internalized insulin is dissociated from its receptor[1,2] and both are properly sorted.[2,17] The carboxylic ionophore monensin binds Na^+, K^+, and protons and acts as a diffusional carrier mediating one-for-one cation exchange, and thereby accomplishes partial equilibration of ions with which it interacts.[8] As a result of these actions, one of the main effects of monensin is the inhibition of acidification of intracellular structures, including the endosomal apparatus.[8] Other studies have shown that monensin is able to cause a marked impairment of insulin degradation in cell systems[18-21] and in endosomes,[22] insulin–insulin receptor complex dissociation,[15,19-21] and insulin receptor recycling.[23] Nevertheless, this study provides the first direct comparison between the effects of monensin in cells from normals and NIDDM patients, with the aim of providing more insight into the molecular mechanisms that underlie the defects observed in NIDDM. Two main results arise from the studies with monensin. Firstly, in cells from normal controls, monensin induces impairment of the dissociation of the insulin-receptor complex and a variety of perturbations of insulin and insulin receptor processing, including the decrease of insulin degradation and the paralysis of the insulin receptor recycling pathway.

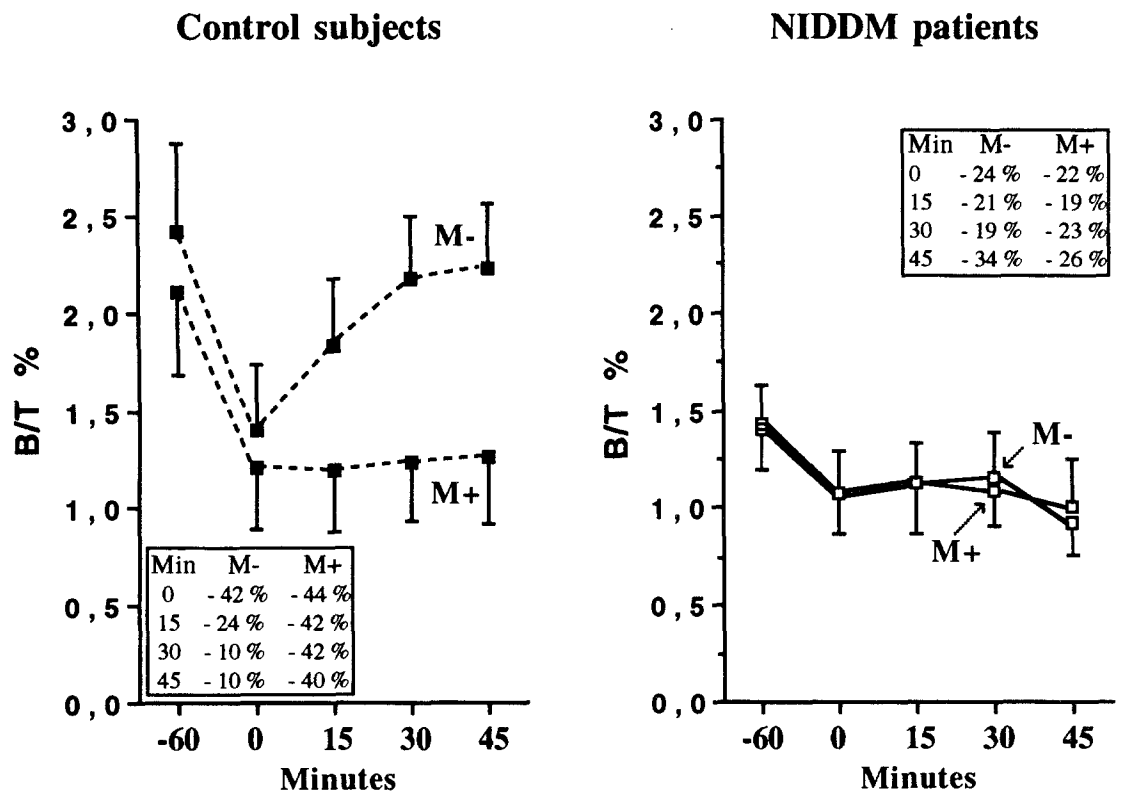

Fig 4. Insulin receptor internalization and recovery of cell-surface insulin binding (B/T) in monocytes from normal subjects and NIDDM patients in the continuous presence (+) or absence (−) of monensin (M). Cells were incubated at 37°C for 60 minutes with 100 nmol/L insulin, acid-washed, and reincubated in insulin-free medium for 45 minutes at 37°C to allow recovery of cell-surface insulin binding. A_{14}-insulin binding was performed at 12°C before (−60 minutes) and after (0 minutes) preincubation with unlabeled insulin and at different times (15, 30, and 45 minutes) after unlabeled-insulin removal. The inserts show % differences for insulin binding measured during the study as compared with the initial binding (−60 minutes) considered as 100%. The recovery of cell-surface insulin binding in control + is significantly different (ANOVA, $P < .05$) compared with that of control M−.

This observation suggests that the inhibition of endosome acidification causes the impairment of dissociation of the insulin-receptor complex, which in turn subtends the decrease of insulin degradation and the blocking of receptor recycling. However, monensin either does not induce or induces only a marginal worsening of the derangements associated with NIDDM. Similarly, when the effects of monensin and diabetes were evaluated on asialoglycoprotein degradation in rat hepatocytes,[24] it was reported that monensin mirrored diabetes-induced effects in normal cells, but had no apparent effect in diabetic cells. Secondly, the results obtained show that NIDDM and monensin induce similar alterations in the intracellular steps of insulin and insulin receptor processing. The only defect present in cells from NIDDM patients but not in monensin-

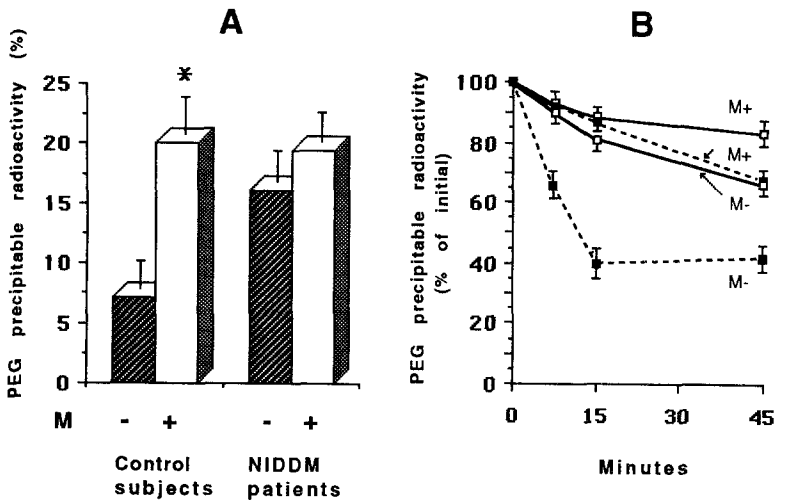

Fig 5. (A) Amount of intracellular PEG-precipitable radioactivity. Monocytes from control subjects and NIDDM patients were incubated in the continuous presence (+) or absence (−) of monensin (M) with A_{14}-insulin at 37°C for 60 minutes, acid-washed, and solubilized to determine the % intracellular PEG-precipitable radioactivity. *$P < .01$ *v* control M−. (B) Time course of the decrease in intracellular PEG-precipitable radioactivity (% of initial). After incubation with A_{14}-insulin, aliquots of cells from control subjects (■) and NIDDM patients (□) incubated in the continuous presence (+) or absence (−) of monensin (M) were resuspended in insulin-free medium at 37°C and solubilized at the indicated times, and intracellular radioactivity was analyzed for the ability to precipitate with PEG. The decrease found in control M+ is significantly different (ANOVA, $P < .05$) compared with control M−.

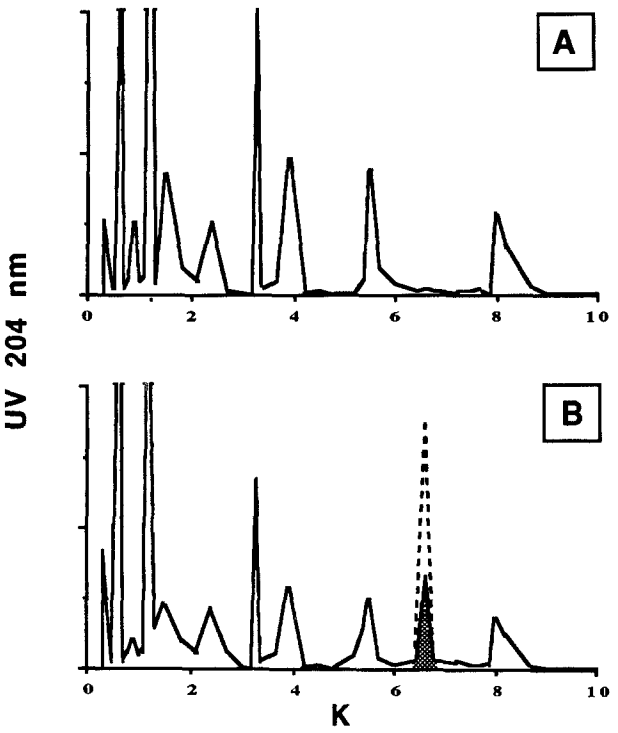

Fig 6. Concentrations of cell-associated monensin. HPLC elution profile of solubilized cells incubated (A) in the absence of monensin and (B) in the presence of monensin. Hatched and dotted peaks represent elution profiles of standard monensin and cell-associated monensin, respectively. The elution patterns and peak heights of monensin were superimposable in cells from normal controls and NIDDM patients.

treated cells is the impairment of internalization of insulin and insulin receptor. Thus, internalization happens via a monensin-insensitive pathway that is not influenced by the subsequent fate of the internalized insulin–insulin receptor complex. Interestingly, a defect of insulin internalization has not been observed in cultured cells from patients with obesity or NIDDM.[7] The similarity of derangements in insulin and insulin receptor processing associated with NIDDM to those that happen as a result of monensin action in normal cells, together with the observation that monensin has little effect in diabetic cells, does not prove but does suggest the existence of similar alterations of the same intracellular mechanism. Clearly, the extent to which the knowledge concerning the mode and site of action of monensin could apply to NIDDM remains speculative. Nevertheless, inhibition of the endosomal acidification process may similarly take place as an effect of monensin and NIDDM. In fact, even if direct evidence is lacking, there are good reasons to assume that NIDDM could be associated with an impairment of intracellular monovalent cation homeostasis,[25-28] which in turn could affect endosomal acidification.

We cannot exclude that other explanations might account for the present results and that monensin and NIDDM act on the same process by different mechanisms of action. For example, the defects observed in NIDDM cells could be linked to a defective insulin receptor kinase activity. Nevertheless, the defective dissociation has been observed in the presence of normal receptor phosphorylation.[7] Moreover, some data suggest that a decreased insulin degradation decreases the rate of insulin dissociation from its receptor inside the cells.[23] Nevertheless, in the latter case with monensin acting by impairing dissociation of the insulin–insulin receptor complex, it could have had an addictive effect on the alteration of insulin degradation already present in NIDDM cells.

Finally, note that the interpretation of results obtained with monensin, as with any other inhibitor, requires the recognition that they are not totally specific. Particularly, monensin has important actions at the level of the Golgi apparatus, and there are data to suggest that it is able to act by inhibiting intracellular transport of secretory proteins.[5] Nevertheless, the role of the Golgi apparatus in the pathway of intracellular insulin processing and recycling of the insulin receptor has not been established. Moreover, it has been found[23] that after exposure of hepatocytes for 30 minutes to insulin and monensin, removal of both agents produced a 50% restoration of cell-surface binding within 10 minutes, whereas no restoration occurred after removal of the hormone with the drug still present. This short time does not seem compatible with a receptor recycling through the Golgi apparatus. Clearly, these are indirect data and do not rule out the possibility that insulin receptor recycling could be routed by the Golgi complex.

In conclusion, this study confirms the existence in cells from NIDDM patients of a derangement of dissociation of the insulin–insulin receptor complex that probably determines the subsequent abnormal sorting and processing of insulin and its receptor. Moreover, the data obtained using monensin suggest that the intracellular mechanism that subtends this derangement could be localized within the endosomal apparatus and consist mainly of a defect in acidification of the endosomal interior. Direct evaluation of the function of the endosomal apparatus of normal and diabetic cells is necessary to address these questions.

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