Effect of Sulfonylurea Treatment on In Vivo Insulin Secretion and Action in Patients with Non-insulin-dependent Diabetes Mellitus

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SUMMARY

The effect of glipizide treatment on diabetic control and on in vivo insulin secretion and action was studied in 20 patients with non-insulin-dependent diabetes mellitus (NIDDM). Patients were examined before and after a minimum of 3 mo treatment. Mean (± SEM) fasting plasma glucose level fell from 264 ± 12 mg/dl to 172 ± 10 mg/dl ($P < 0.001$) after glipizide treatment, and this was associated with a fall in total plasma glucose response to a test meal of approximately 35%. Mean (± SEM) fasting plasma insulin levels increased slightly from 15 ± 2 μU/ml to 18 ± 2 μU/ml following sulfonylurea treatment, and the total plasma insulin response to the test meal increased by 63%. However, there was no correlation ($r = -0.20$) between the increase in plasma insulin response and the fall in plasma glucose levels that occurred as the result of sulfonylurea therapy. Glipizide treatment also led to enhanced in vivo insulin action, whether measured by the insulin clamp technique ($P < 0.001$) or the insulin suppression test ($P < 0.02$). Furthermore, in this instance there was a significant correlation ($r = 0.69$, $P < 0.001$) between the enhanced insulin action and the improvement on diabetes control. Thus, chronic therapy with glipizide, a new sulfonylurea agent, led to increased in vivo insulin secretion and insulin action. These results lend direct support to the assumption that sulfonylurea compounds have a substantial extrapancreatic effect on glucose homeostasis, and suggest that this effect contributes to the therapeutic efficacy of these drugs. **DIABETES *31*:307–312, April 1982.**

Several publications over the past 15 yr have indicated that plasma insulin levels in patients being successfully maintained on chronic sulfonylurea therapy were no higher than pretreatment levels.[1–5] These observations led to the obvious suggestion that the efficacy of these compounds in the treatment of diabetes might be related to their ability to augment in vivo insulin action, i.e., an extrapancreatic effect.[5] Although this hypothesis appears to be a reasonable one, there is relatively little direct experimental support for this formulation. Indeed, we are only aware of one report[6] in which the effect of sulfonylurea therapy on both insulin secretion and in vivo insulin action has been measured in patients with non-insulin-dependent diabetes mellitus (NIDDM) treated for over 1 mo. In this study, insulin secretion was estimated by determining the plasma insulin response to oral glucose and insulin action by performing an intravenous insulin tolerance test. Both insulin secretion and action seemed to improve within 6 wk of glipizide therapy. Although the authors felt that the effect on insulin action played an important role in the therapeutic efficacy of glipizide, a detailed analysis of the relative roles of the increases in insulin secretion and action was not conducted. Since we feel the issue to be an important one, the current investigation was carried out to quantitate the effect of several months of glipizide treatment on insulin secretion and in vivo insulin action in patients with NIDDM. However, our study differs in several regards from the earlier report. In the first place, we have quantitated the effect of glipizide on insulin secreton by determining the plasma insulin response to a test mixed-meal. Secondly, we have quantitated in vivo insulin action by two methods: the insulin suppression test[7] and the insulin clamp technique.[8] With these approaches, we have been able to demonstrate that chronic glipizide treatment led to increases in the insulin response to the test meal, as well as to an enhancement of in vivo insulin action. It seems reasonable to conclude that both of these changes contributed to the therapeutic efficacy of the drug.

METHODS

PATIENTS

Patients with NIDDM were admitted to the study if they had a fasting plasma glucose concentration >150 mg/dl. The study population consisted of 10 females and 10 males, with

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a mean age of 59 yr (range 50–69 yr). The mean duration of diagnosed diabetes was 6.4 yr, and the mean body mass index [wt. (kg)/ht (m)^2] was 28.3 kg/m^2 (range 24.0–36.0 kg/m^2). Subjects were either previously treated with diet alone, or with an oral hypoglycemic agent. None had been treated with insulin, and all hypoglycemic medication was discontinued at least 3 wk before admission. There was no evidence of significant renal, hepatic, or thyroid disease by history, physical examination, or chemistry survey panel.

PROTOCOL

Patients were admitted to the Stanford University General Clinical Research Center, and their informed consent was obtained. Isocaloric formula diets were consumed as three meals, consisting of 1/5 of total calories for breakfast (0800), and 2/5 each for lunch (1200) and dinner (1800). The following measurements were made, both before and after glipizide treatment.

Fasting plasma glucose and insulin concentrations. At least four blood samples were obtained at 0800 h, before breakfast, for determination of fasting plasma glucose and insulin concentrations.

Meal tolerance tests. Glucose tolerance and insulin secretion were assessed by measuring plasma glucose and insulin concentration in response to the noon meal on two occasions during each hospitalization. Samples were obtained at 1200 h, before the mixed-meal, and at 1230, 1300, 1400, and 1500 h. The glucose and insulin responses were also quantified as the total area subtended by the plasma glucose and insulin concentrations. These data are expressed as mg/dl · h for glucose response and μU/ml · h for the insulin response.

Insulin resistance. In vivo insulin action, before and after glipizide treatment, was assessed by two techniques.

(a) Insulin clamp. Since this method has been previously described in detail,[9] only the general procedure will be outlined. Blood samples were obtained every 5 min from an indwelling catheter in a hand vein. The hand was kept in a radiant warmer at 70°C to provide arterialized samples. Plasma was immediately separated in a Beckman microfuge (Beckman Model S, Beckman Instruments, Fullerton, California), and glucose determined in triplicate using a Beckman Glucose Analyzer II (Beckman Instruments). After establishing the baseline plasma glucose concentration, a primed, continuous infusion of insulin at 42.6 mU/m^2/min was started. Plasma glucose was determined every 5 min. At 4 min after the start of the insulin infusion, a variable infusion of glucose was administered in the amount required to maintain basal glucose concentrations. The mean coefficient of variation (CV) of the plasma glucose levels during the clamp studies was ±4%, with no variations greater than ±8%. The amount of glucose metabolized (M) between 20 and 120 min of the study was computed from the amount of glucose infused, with corrections made for urinary glucose loss and changes in glucose pool size.[9]

Although M is assumed to provide a method to compare in vivo insulin action of different individuals, there is a potential drawback to its use in the present study. Basal plasma glucose concentrations differed as the result of glipizide treatment. Since the rate of glucose utilization is related to glucose concentration,[10] M cannot be used to compare in vivo insulin action of individuals with different basal plasma glucose concentrations. One way to avoid this dilemma is to determine the efficiency of glucose utilization, i.e., the glucose metabolic clearance rate (MCR). MCR can be calculated by simply dividing M by the plasma glucose level during the clamp, and should provide a means of comparing the efficiency of in vivo insulin action of individuals with different basal plasma glucose concentrations.

To validate this use of MCR, a pair of special insulin clamps were performed in seven patients. One clamp was performed in which basal plasma glucose level was maintained in the usual fashion for a period of 3 h, and determination of M and MCR made during the last hour (basal glucose). During the second study, the plasma glucose concentration was allowed to fall for 2 h, and the variable glucose infusion was adjusted to maintain the plasma glucose at the new lower value for the third hour (reduced glucose). Glucose utilization and metabolic clearance rate were then computed for the third hour. Four patients were studied in this fashion before glipizide treatment, and three patients were studied after chronic therapy. The results were the same in both groups, and are presented together in Table 1. These data demonstrate that M will change in the same patient as a function of plasma glucose concentration, but that MCR will remain constant under the conditions of these studies. Thus, we have used MCR to compare in vivo insulin action in patients before and after glipizide treatment. These results are somewhat different than the recently published demonstration by Verdonk et al.[11] that MCR decreased as plasma glucose was increased. However, their studies were conducted during somatostatin suppression of endogenous insulin secretion, at insulin levels during the clamp studies of approximately 18 μU/ml, and at plasma glucose levels that varied from 60–165 mg/dl. We would suggest that the major reason for the discrepancy is the fact that the usual clamp studies are conducted at elevated levels of insulin, i.e., approximately 100 μU/ml, not at basal levels of 18 μU/ml. Be that as it may, their observations do not detract from our use of glucose MCR to assess in vivo insulin action under the conditions of our study.

(b) Insulin suppression test. An infusion of epinephrine, propranolol, glucose, and insulin was administered as previously described.[7] After an overnight fast, patients were infused with insulin (80 mU/min), glucose (6 mg/kg/min), epinephrine (6 μg/min), and propranolol (80 μg/min), for 150 min. Under these conditions, endogenous insulin secretion is inhibited, and glucose uptake is stimulated by the

TABLE 1
Effect of variations in steady-state plasma glucose concentrations on glucose utilization and clearance*

	Basal glucose	Reduced glucose
Plasma glucose concentration (mg/dl)	221 ± 20	121 ± 8
Plasma insulin concentration (μU/ml)	97 ± 6	95 ± 4
Glucose utilization M (mg/kg/min)	4.33 ± 0.61	2.45 ± 0.25
Glucose clearance MCR (ml/kg/min)	1.90 ± 0.35	2.05 ± 0.21

* Data expressed as mean ± SEM.

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infused insulin. Steady-state plasma glucose (SSPG) and insulin (SSPI) levels were attained by 90 min (CV < 10%), and samples were obtained for determination of glucose and insulin concentrations every 5 min for the last half hour of the study. Since similar SSPI concentrations were achieved in all patients, the SSPG level was a direct measurement of the ability of insulin to promote disposal of a glucose load in different subjects.

The tests were performed in random fashion, and carried out on every other day. After these initial studies, glipizide treatment was initiated. Patients were treated with 5–15 mg/day in divided doses at the outset. Diet and exercise were not changed. They were then seen at weekly intervals as outpatients. Subjects were interviewed for any adverse effects and blood was obtained for fasting plasma glucose and insulin concentrations. Doses were increased until a maximally effective dose was determined, or a maximum dose of 50 mg/day was prescribed. After a minimum of 3 mo of therapy, the patients were readmitted to the Clinical Research Center, and all studies were repeated.

Plasma glucose[12] and insulin[13] concentrations were determined according to previously described methods. Statistical analysis was performed using paired *t* tests and regression analysis from the Statistical Package for the Social Sciences.[14]

RESULTS

Glipizide was well tolerated by all subjects. Three patients complained of mild paresthesias shortly after starting the medication, but these symptoms resolved within 2–3 wk. There were no hematologic or hepatic complications from therapy. Although previous reports have shown therapeutic doses in the range of 5–20 mg/day,[15] the mean (± SD) dose in this study was 36 ± 12 mg/day, with 80% of the patients requiring 30 mg or more for optimal therapeutic effect. There was one primary failure among the 20 subjects. The data for this subject has been included in subsequent analyses except where indicated.

The effect of treatment on plasma glucose and insulin concentrations was determined after an overnight fast, as well as in response to the noon mixed-meal. The results of these studies are shown in Figure 1. Mean (± SEM) plasma glucose concentration was 264 ± 12 mg/dl before therapy, and decreased significantly to 172 ± 10 mg/dl ($P < 0.001$) after a minimum of 3 mo treatment. Postprandial glycemic excursions were similarly improved. Mean preprandial plasma glucose concentration was 304 ± 15 mg/dl before therapy, and this value rose to a maximum of 408 ± 20 mg/dl 2 h after the meal. Following therapy there was a fall in mean pre- and postprandial plasma glucose concentrations to 184 ± 14 mg/dl and 262 ± 15 mg/dl, respectively. Mean plasma glucose levels at each time point were significantly lower ($P < 0.001$) after treatment, and the total glucose response (determined as the area under the response curve) fell from 1139 ± 53 mg/dl · h to 751 ± 43 mg/dl · h ($P < 0.001$).

The effect of glipizide treatment on plasma insulin concentrations is shown in the right hand panel of Figure 1. Plasma insulin concentrations after an overnight fast increased slightly, but significantly ($P < 0.02$), as a result of treatment (15 ± 2 μU/ml vs. 18 ± 2 μU/ml). However, the change in insulin response to the noon meal was of far

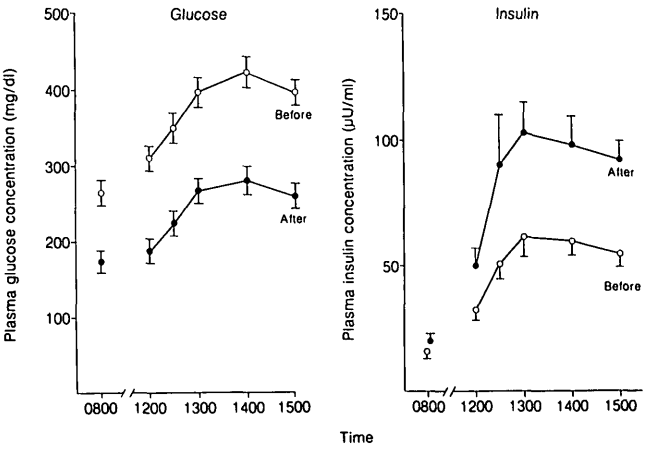

FIGURE 1. Mean (±SEM) plasma glucose and insulin concentration before breakfast (0800), before lunch (1200), and for 3 h after lunch in 20 patients before and after glipizide treatment.

greater magnitude. Plasma insulin levels before treatment increased from a preprandial value of 30 ± 5 μU/ml to a maximum of 61 ± 10 μU/ml at 1 h. Sulfonylurea therapy resulted in significantly greater insulin concentrations at each time point ($P < 0.01$), with mean preprandial and 1 h postprandial values increasing to 47 ± 9 μU/ml and 95 ± 15 μU/ml, respectively. In addition, the total plasma insulin response increased by 63% ($P < 0.001$) following treatment. It is interesting to note that these subjects were capable of increasing their insulin response to the noon mixed-meal even before therapy. It should also be pointed out that the plasma insulin levels in these patients were, at least in absolute terms, quite comparable to those of nonobese subjects with normal glucose tolerance previously reported from our laboratory.[16] Thus, they were not absolutely insulin-deficient.

To evaluate the effect of glipizide treatment on in vivo insulin action, we used two techniques: the insulin clamp and the insulin suppression test. The glucose "goal" for each insulin clamp study was the fasting plasma glucose concentration the morning of the study. Given the fact that these levels vary between the two studies as a function of the efficacy of treatment, and previous observations that the plasma glucose concentration modulates glucose utilization rate,[10] we could not use M (glucose utilization rate) as a means of comparing patients before and after therapy. Instead, we compared patients on the basis of the glucose metabolic clearance rate (MCR), i.e., a measure of the efficacy of insulin-stimulated glucose utilization. The results of these studies are seen in Figure 2. There was no statistically significant difference in plasma insulin concentrations achieved during the clamp studies before and after therapy (96 ± 5 μU/ml and 106 ± 5 μU/ml). The metabolic clearance rate increased from a pretreatment value (mean ± SEM) of 1.24 ± 0.11 mg/kg/min to a posttreatment value of 1.87 ± 0.16 mg/kg/min. This 52% increase in MCR was highly significant ($P < 0.001$).

An improvement in insulin resistance was also demonstrated using the insulin suppression test. Since this procedure results in alpha-adrenergic stimulation, it was only performed in patients without hypertension or cardiovascular disease. The results of these studies in nine patients are il-

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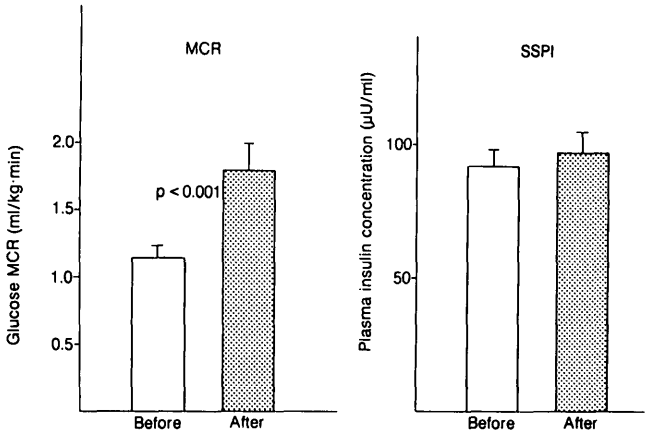


FIGURE 2. Mean (±SEM) values for glucose metabolic clearance rate (MCR) and steady-state plasma insulin (SSPI) concentrations observed during the insulin clamp studies performed in 20 patients before and after glipizide treatment.

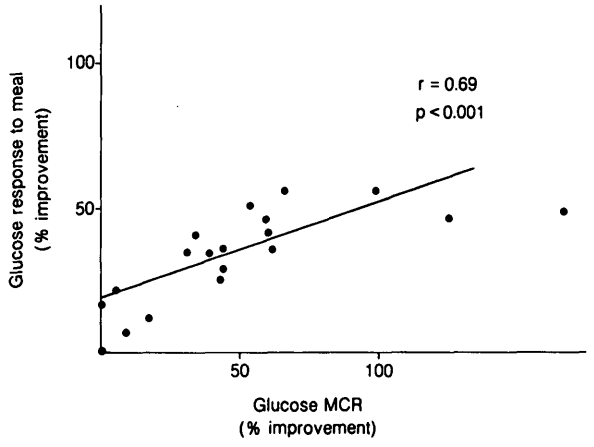


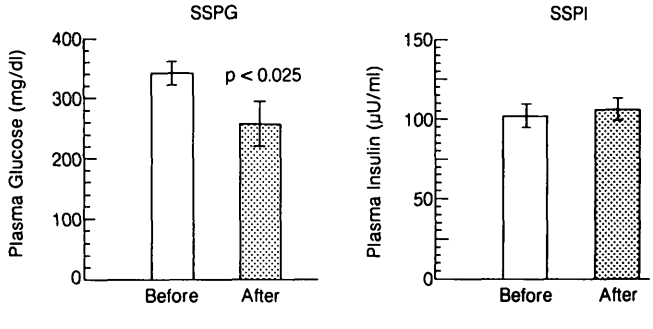
FIGURE 4. Relationship between the percent improvement in the glucose response to the meal and glucose metabolic clearance rate (MCR) in patients treated with glipizide.

lustrated in Figure 3. Mean (±SEM) steady state plasma glucose concentration, a direct measure of insulin resistance, decreased by 60% from 342 ± 20 mg/dl to 258 ± 37 mg/dl ($P < 0.02$) following treatment. The insulin concentrations during the infusions were identical before and after therapy (103 ± 7 μU/ml and 106 ± 7 μU/ml, respectively). Although the fasting plasma glucose concentrations are different in the two studies, previous results from our laboratory have indicated that the SSPG results are independent of variations in plasma glucose pool size.[17] The insulin suppression test results are similar to those obtained with the insulin clamp, and document an improvement in in vivo insulin action.

Chronic therapy with glipizide resulted in improved diabetic control, with increases in both insulin secretion and insulin action. In an attempt to gain insight into the relative role of these two variables in the improved glucose tolerance, the data were subjected to regression analysis. Ratios of posttreatment to pretreatment glucose and insulin areas during the meal tolerance tests were used to quantitate improvement in carbohydrate tolerance and insulin secretion. Change in MCR was computed as a ratio of posttreatment to pretreatment values, and used as an index of improvement in insulin action. The one primary failure was excluded from this analysis.

Despite a wide range in the increases in insulin responses following treatment (0–210%), there was no significant ($r = -0.20$) correlation between the change in glucose tolerance and change in insulin response (data not shown). The correlation between improvement in glucose response and MCR is shown in Figure 4. There was a highly significant correlation between these two variables ($r = 0.69$, $P < 0.001$).

FIGURE 3. Mean (±SEM) steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations observed during the insulin suppression studies performed in nine patients before and after glipizide treatment.



DISCUSSION

In this study we have used two methods, the insulin clamp and the insulin suppression test, to document the fact that the hypoglycemic effect of chronic sulfonylurea therapy is associated with an enhancement of in vivo insulin action. Although estimates of insulin action obtained by the two methods have been shown to correlate closely,[9] demonstration that two such disparate methods lead to similar results greatly augments our ability to generalize from these data. When taken together with previous results in man,[6] dog,[18,19] and mouse,[20,21] there seems to be substantial direct evidence for the view that sulfonylurea administration leads to a potentiation of in vivo insulin action. Furthermore, there was a significant correlation ($r = 0.69$) between the drug-induced improvement in glucose tolerance and the enhancement of insulin action. Thus, it seems reasonable to suggest that the efficacy of sulfonylurea therapy may, at least to some extent, be related to its ability to augment the effect of the endogenous insulin present in patients with NIDDM. However, it is essential that a distinction be made between such patients and those diabetics who are absolutely insulin-deficient. Whether sulfonylurea therapy will augment insulin action in insulinopenic patients treated with exogenous insulin remains to be determined. However, there is evidence from studies of pancreatectomized dogs[22,23] that this might also occur.

On the other hand, our results do not necessarily mean that the primary therapeutic action of sulfonylurea compounds is mediated by an improvement in in vivo insulin action. In the first place, it is obvious that the insulin response to meals was significantly augmented by sulfonylurea therapy. Secondly, since we only measured peripheral plasma insulin concentrations, our data have minimized the effect of glipizide on insulin secretion by not being able to sample portal vein blood. Indeed, sulfonylurea treatment may lead to differences in hepatic extraction of insulin, which could

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account for our inability to document a significant relationship between sulfonylurea-induced changes in peripheral plasma insulin levels and therapeutic response. Thirdly, it is possible that the sulfonylurea-induced increases in insulin secretion led to the improvement in insulin action. Thus, we have demonstrated that insulin resistance developed in normal dogs made severely insulin deficient with alloxan, and that in vivo insulin sensitivity was restored to normal following insulin replacement.[24] Further evidence of the complex relationship between insulin secretion and insulin resistance can be seen from the results of Ginsberg and Rayfield,[25] who indicated that insulin therapy improved the in vivo action of insulin in some proportion of patients with NIDDM. Given all of these considerations, it is obviously impossible to decide which action of glipizide, the increase in insulin secretion or the enhancement of insulin action, was most responsible for the improvement of glucose tolerance. Indeed, it may well be that this varies from patient to patient.

At the present, there is considerable uncertainty as to the manner in which sulfonylureas potentiate insulin action. Plasma insulin levels are often elevated in patients with NIDDM, and it has been suggested that this may lead to a "downregulation" of the number of insulin receptors and insulin resistance.[26] If plasma insulin levels were to fall as a result of sulfonylurea therapy, as is sometimes the case,[1-4] one might argue that this would lead to an increase in number of insulin receptors on circulating monocytes of patients with NIDDM,[27,28] as well as hepatocytes of normal mice.[20] However, the observation by Prince and Olefsky[29] that the addition of a sulfonylurea to cultured fibroblasts led to an increase in number of insulin receptors suggests that a sulfonylurea-induced change in receptor number may be a direct effect, and need not be mediated via a change in circulating insulin level. On the other hand, it has recently been shown[30] that adipocytes obtained from adipose tissue maintained for more than 20 h in the presence of a sulfonylurea have an increased response to the stimulating effects of insulin. Since there was no associated change in either number or affinity of insulin receptors, Maloff and Lockwood[30] suggested that sulfonylurea compounds alter postreceptor insulin action, and their hypoglycemic effect might be related to stimulation of insulin-stimulated hexose transport. The reason for these differences are not clear, and may well represent tissue-specific variations in response to sulfonylureas. Obviously, additional information will be needed in order to define the mechanism by which sulfonylureas enhance in vivo insulin action. However, since it appears that the insulin resistance of patients with NIDDM cannot be entirely explained on the basis of decreased insulin binding,[31] demonstration that a sulfonylurea can potentiate insulin-stimulated glucose utilization distal to the receptor is of considerable importance.

Finally, the role played by increased insulin secretion in the mechanism of action of sulfonylurea compounds is extremely difficult to assess. In the first place, it is obvious that improvement of glucose tolerance can occur in the absence of a sustained increase in plasma insulin levels.[1-4] Secondly, given the evidence that an increase in plasma insulin concentration can lead to a decrease in the number of insulin receptors,[26] a sustained increase in circulating insulin levels may not be totally beneficial. On the other hand, to the degree that the glucose intolerance and/or the insulin resistance of patients with NIDDM[32] is secondary to hypoinsulinemia, an increase in plasma insulin levels should be of benefit. Thus, it is apparent that there is a need to learn more about the relationship between improved diabetic control with sulfonylurea compounds and the effect of these drugs on the beta-cell.

In conclusion, treatment of patients with NIDDM with a new, second generation sulfonylurea compound, led to a significant improvement in both fasting and postprandial glucose levels. The improvement in glucose homeostasis was associated with an apparent increase in both insulin secretion and action, and it seems reasonable to assume that both of these effects contributed to the therapeutic efficacy of glipizide. The relative role played by the sulfonylurea-induced enhancement of in vivo insulin action in the improved diabetic control, as well as the mechanism by which sulfonylureas potentiate insulin action, remains to be clarified.

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