Gliclazide Therapy Is Associated with Potentiation of Postbinding Insulin Action in Obese, Non-insulin-dependent Diabetic Subjects

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SUMMARY

Six obese, non-insulin-dependent diabetic subjects were studied before and 3 mo after treatment with the sulfonylurea gliclazide, 40–80 mg b.i.d. Fasting plasma glucose fell significantly from 13.4 ± 1.6 (SEM) to 8.6 ± 1.2 mmol/L, accompanied by a significant reduction from 40.6 ± 3.7 to 29.8 ± 2.8 mM · h of the plasma glucose response to 75 g oral glucose. Fasting plasma insulin showed a nonsignificant increase from 24.8 ± 2.0 to 31.3 ± 2.3 mU/L. The percent specific binding of tracer ^{125}I-insulin to erythrocytes and monocytes did not change significantly (from 9.8 ± 1.7 to 8.5 ± 0.7 for erythrocytes and 1.7 ± 0.3 to 1.6 ± 0.4 for monocytes). Glucose utilization was measured at three levels of insulin infusion (40, 100, and 300 mU/kg/h) by the euglycemic clamp technique. Overall there was a significant ($P < 0.05$) increase in the disappearance rate (R_d) and metabolic clearance rate (MCR_g) for glucose at the two higher insulin infusion rates (MCR_g: 3.3 ± 0.7 to 5.1 ± 0.7 and 5.9 ± 0.9 to 7.9 ± 0.9 ml/kg/min), but not at the lowest infusion rate (MCR_g: 3.6 ± 0.8 to 3.3 ± 0.6). Thus, the chronic hypoglycemic effect of gliclazide in obese diabetic subjects was associated with an improvement in insulin-mediated glucose utilization at high plasma insulin concentrations. This enhanced effect of insulin after gliclazide treatment was not accompanied by increased monocyte or erythrocyte insulin binding, which suggests that it was due to potentiation of postbinding insulin-sensitive pathways. DIABETES 1985; 34:241–45.

There is increasing evidence that sulfonylurea drugs, in addition to their well-studied effect to stimulate insulin secretion,[1] exert long-term hypoglycemic actions that are partly extrapancreatic and related to potentiation of the bioeffects of insulin.[2–5] Some studies in vivo[6,7] have suggested that this extrapancreatic effect is associated with an increase in cellular insulin receptor numbers, but give no indication whether these receptor changes are a direct or indirect effect of sulfonylureas. Exposure of cultured cells to sulfonylureas has yielded variable results. Recent studies, in vitro, have shown no effect of sulfonylureas on receptor binding[8–10] but an enhancement of maximal insulin-stimulated glucose uptake[8] and lipogenesis,[9] suggesting a direct effect of sulfonylureas on the postbinding pathways of insulin action.

We aimed to study, in symptomatic obese, non-insulin-dependent diabetic subjects, the mechanisms of a possible extrapancreatic hypoglycemic action of the sulfonylurea gliclazide.[11] Erythrocytes and monocytes were used to assess insulin receptor status. To determine if gliclazide therapy was associated with potentiation of insulin action, insulin dose responses were measured in vivo using the euglycemic clamp technique.[12]

MATERIALS AND METHODS

Informed consent to the study was obtained from six obese, non-insulin-dependent diabetic subjects whose blood sugars were inadequately controlled on diet alone. The subjects were five men and one woman aged 53–67 yr and 110–170% of ideal body weight. Their mean fasting plasma glucose concentration before therapy was 13.4 ± 1.6 (SEM) mmol/L. They were commenced on oral gliclazide, in doses ranging from 40 to 80 mg twice daily, depending on the response of their fasting plasma glucose measured every 2 wk, and were instructed not to alter their pattern of food intake. Before and 3 mo after commencing gliclazide the following measurements were made: (1) plasma glucose and insulin both fasting and during a 75-g oral glucose tolerance test (OGTT); (2) insulin receptor binding assays on freshly isolated circulating erythrocytes and monocytes[13] using A14-monoiodoinsulin kindly supplied by Dr. F. Alford (the

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coefficients of variation of tracer binding were 3.1% and 7.6%, respectively); and (3) insulin dose responses in vivo, using the euglycemic clamp technique to measure insulin-mediated glucose utilization at steady state at three levels of constant insulin infusion (40, 100, and 300 mU/kg/h) as previously described.[14] Gliclazide dosages were omitted only on the day of each of these procedures.

In the euglycemic clamp, the blood glucose level was maintained at 4.5 mmol/L at each of three levels of constant insulin infusion by adjusting the rate of glucose infusion according to a computer algorithm[12] based on 5-min measurements of blood glucose using a Yellow Springs Analyzer (Yellow Springs, Ohio). In these studies, the mean of the coefficients of variation of the steady-state plasma glucose levels at plateau was 3.5 ± 0.3%. The mean coefficient of variation for the steady-state plasma insulin concentration was 10.5 ± 1.1%. During the first two periods of insulin infusion, a constant infusion of ^{3}H-3-glucose (approximately 15 μCi/h), after an initial bolus dose, was given to determine the rate of glucose appearance (R_a) as previously described.[14] At least 90 min was allowed for equilibration. In the first four subjects, hepatic glucose production was suppressed by insulin at 100 mU/kg/h and, therefore, in the last two subjects the tracer infusion was used only during the first period when insulin was infused at 40 mU/kg/h. For technical reasons, in subject D no data were obtained at the lowest infusion rate after gliclazide therapy. Steady state was attained in all periods except one (mean coefficient of variation of plasma specific activity at plateau, 5.1 ± 0.7%), and R_a was calculated using the formula $R_a = F/SA$, where F is the infusion rate of tracer and SA is the plateau specific activity. At steady state, the hepatic glucose production (HGP) is equal to R_a minus the glucose infusion rate, and R_d is equal to R_a. In the one period in which steady state was not attained, R_a and R_d were calculated using the Steele equation with 0.65 as the pool fraction.[15] At the highest of the three infusion rates, HGP is suppressed and R_d is equal to the rate of glucose infusion. The metabolic clearance rate of glucose (MCR_g) was calculated by dividing R_d by the plasma glucose concentration.

The statistical significance of changes was determined using the paired *t*-test.

RESULTS

Data obtained from studies before and after 3 mo of gliclazide therapy, apart from the glucose clamps, are shown in Table 1. Data from the glucose clamp studies are presented in Table 2.

During therapy with gliclazide, fasting plasma glucose fell from 13.4 ± 1.6 (SEM) to 8.6 ± 1.2 mmol/L ($P < 0.05$). No improvement was seen in one subject (C), who we suspect was noncompliant. Fasting plasma insulin showed a nonsignificant increase from 24.8 ± 2.0 to 31.3 ± 2.3 mU/L. Oral glucose tolerance, as measured by the area under the plasma glucose curve after 75 g of oral glucose, improved significantly from 40.6 ± 3.7 (SEM) to 29.8 ± 2.8 mM · h

TABLE 1
Plasma glucose and insulin concentrations fasting and after 75 g oral glucose, and specific insulin binding to monocytes and erythrocytes (at tracer-only point).

	A	B	C	D	E	F	Mean ± SEM
Before therapy							
Fasting plasma glucose (mmol/L)	8.0	10.6	14.4	16.2	18.5	12.4	13.4 ± 1.6
Fasting plasma insulin (mU/L)	16.8	33.2	18.0	24.0	42.0	15.0	24.8 ± 2.0
Specific insulin binding (%)							
Erythrocytes	7.5	6.2	10.0	5.9	13.0	12.1	9.8 ± 1.7
Monocytes	1.6	1.2	1.0	1.8	2.6	2.0	1.7 ± 0.3
OGTT							
Glucose/insulin at (min)							
0	8.0/17	10.6/33	14.4/18	16.2/24	18.5/42	12.4/15	
30	12.0/22	13.7/43	18.0/37	19.5/31	26.6/56	14.9/16	
60	19.0/25	16.7/93	21.0/28	25.3/30	28.4/66	18.1/44	
90	24.6/28	17.9/105	23.9/32	27.6/48	30.7/54	19.2/48	
120	22.1/25	17.0/102	23.1/34	25.8/37	30.5/45	20.3/40	
Glucose area (total)	35.0	31.0	40.0	46.0	55.0	34.0	40.6 ± 3.7
Insulin area (incremental)	14.0	88.0	26.0	22.0	26.0	38.0	35.6 ± 11.0
After therapy							
Fasting plasma glucose (mmol/L)	6.5	6.5	14.1	11.8	6.4	8.3	8.6 ± 1.2*
Fasting insulin (mmol/L)	20.0	44.0	28.0	38.0	21.0	14.0	31.3 ± 2.3
Specific insulin binding (%)							
Erythrocytes	8.0	7.2	8.5	7.3	8.5	10.2	8.5 ± 0.7
Monocytes	3.6	0.8	0.8	1.2	1.8	1.0	1.6 ± 0.4
OGTT							
Glucose/insulin at (min)							
0	6.5/20	6.5/44	14.1/28	11.8/38	6.4/21	8.3/14	
30	9.6/39	11.2/125	16.0/37	14.8/63	15.7/62	11.1/25	
60	12.5/47	13.2/126	20.8/39	18.9/82	16.0/43	11.9/23	
90	14.9/65	14.1/156	25.0/69	20.5/82	16.2/74	14.6/27	
120	13.4/55	14.7/185	25.0/61	19.2/97	16.5/61	16.3/35	
Glucose area (total)	23.0	24.0	40.0	35.0	30.0	25.0	29.8 ± 2.8*
Insulin area (incremental)	54.0	172.0	39.0	73.0	69.0	22.0	71.4 ± 21.5

Results in the six subjects (A–F) are shown before and after 3 mo of oral gliclazide therapy.
*$P < 0.05$ by paired *t*-test.

TABLE 2
Mean plateau data from euglycemic clamps in subjects A–F

Pre- and posttherapy		A	B	C	D	E	F	Mean ± SEM
Period 1								
Plasma glucose	Pre	5.1	6.5	5.1	4.9	6.5	5.5	5.7 ± 0.32 (5)
(mmol/L)	Post	4.1	5.5	5.0	—	5.7	6.3	5.3 ± 0.37 (5)
Plasma insulin	Pre	173	75	105	183	37	47	87 ± 24 (5)
(mU/L)	Post	61.5	68	81	—	54	58	64 ± 5 (5)
R_d and (HGP)	Pre	8.5 (−1.7)	17.6 (9.2)	26.9 (6.0)	19.5 (7.3)	17.0 (13.5)	32 (18.5)	20.4 ± 4.1 (5)
(μmol/kg/min)	Post	8.7 (−0.5)	16.2 (8.2)	11.2 (6.0)	—	22.0 (0)	31 (1)	17.8 ± 4.0 (5)
MCR_g	Pre	1.6	2.7	5.3	4.0	2.6	5.9	3.6 ± 0.8 (5)
(ml/kg/min)	Post	2.1	2.9	2.2	—	4.3	4.9	3.3 ± 0.6 (5)
Period 2								
Plasma glucose	Pre	5.5	5.9	4.7	5.0	5.6	5.7	5.4 ± 0.21
(mmol/L)	Post	4.6	5.5	4.8	4.4	4.5	5.2	4.8 ± 0.18
Plasma insulin	Pre	430	125	210	326	87	118	216 ± 56
(mU/L)	Post	147	142	427	158	72	151	183 ± 51
R_d and (HGP)	Pre	13.3 (−1.9)	21.5 (−0.8)	18.5 (0)	28.7 (2.5)	4.8 (—)	17.3 (—)	17.3 ± 3.3
(μmol/kg/min)	Post	16.1 (0)*	33.3 (0.2)	21.7 (−3.4)	30.9 (1.6)	20.2 (—)	27.6 (—)	25.0 ± 2.7†
MCR_g	Pre	2.3	3.6	4.0	5.8	0.9	3.1	3.3 ± 0.7
(ml/kg/min)	Post	3.5	6.1	4.5	7.0	4.5	5.3	5.1 ± 0.7†
Period 3								
Plasma glucose	Pre	5.9	6.0	4.5	5.2	4.8	4.6	5.2 ± 0.27
(mmol/L)	Post	4.6	5.3	5.4	4.7	4.7	4.4	4.9 ± 0.16
Plasma insulin	Pre	1832	698	511	2475	325	418	1043 ± 364
(mU/L)	Post	543	638	854	1340	317	609	717 ± 143
R_d	Pre	22.5	49.7	32.8	39.1	12.0	27.1	30.5 ± 5.4
(μmol/kg/min)	Post	29.2	52.1	36.7	37.5	27.8	42.6	37.7 ± 3.7†
MCR_g	Pre	3.8	8.1	7.3	7.5	2.5	5.9	5.9 ± 0.9
(ml/kg/min)	Post	6.3	9.8	6.8	8.0	5.9	9.7	7.9 ± 0.9†

Mean data from euglycemic clamps on the six subjects (A–F) at three levels of insulin infusion (periods 1, 40; 2, 100; and 3, 300 mU/kg/h) before and after 3 mo of gliclazide therapy. Each data point is the mean of four measurements at 10-min intervals during each steady-state plateau. R_d, glucose disappearance rate; HGP, hepatic glucose production; and MCR_g, metabolic clearance rate of glucose. For technical reasons, no data were obtained in period 1 for subject D.
*Steady state not attained.
†$P < 0.05$ by paired *t*-test.

($P < 0.05$) after gliclazide treatment. No improvement was seen in the subject whose fasting plasma glucose was unchanged. Five of the six subjects showed an increase in the incremental insulin area after oral glucose (35.6 ± 11 to 71.4 ± 21.5 mU/L · h). One subject (A) lost 2 kg weight; the others gained an average of 4.5 ± 0.5 kg.

The specific binding of tracer ^{125}I-insulin to erythrocytes and monocytes showed a nonsignificant decrease after therapy with gliclazide (from 9.8 ± 1.7 to 8.5 ± 0.7% for erythrocytes and from 1.7 ± 0.3 to 1.6 ± 0.4% for monocytes). Binding to monocytes decreased in all subjects except the one who lost weight. On analysis of the full binding curves, there was no significant change in the 50% inhibition dose after gliclazide therapy (erythrocytes: 4.7 ± 1 to 4.8 ± 1.7 ng/ml; monocytes: 5.3 ± 1 to 3.8 ± 0.5), which indicates that there was no consistent effect of therapy on insulin receptor affinity.

Five of the six subjects showed an improvement in insulin action in vivo. The subject who showed no improvement was the one who also showed no improvement in fasting plasma glucose or oral glucose tolerance. Overall, there was a significant increase ($P < 0.05$) in both the R_d and the MCR_g at the two higher insulin infusion rates (R_d: 17.3 ± 3.3 to 25.0 ± 2.7 and 30.5 ± 5.4 to 37.7 ± 3.7 μmol/kg/min; MCR_g: 3.3 ± 0.7 to 5.1 ± 0.7 and 5.9 ± 0.9 to 7.9 ± 0.9 ml/kg/min). The mean increases in MCR_g at all three insulin infusion rates correlated better with the decreases in fasting plasma glucose ($r = 0.81$, $P = 0.05$) than with the increases in incremental insulin area after oral glucose ($r = 0.21$). The steady-state plasma insulin concentrations at each of the three insulin infusion rates were not significantly different between the two treatments (before gliclazide: 87 ± 24, 216 ± 56, and 1043 ± 364; after gliclazide: 64 ± 5, 183 ± 51, and 717 ± 143 mU/L). The plateau plasma glucose concentrations were on the average 0.4 mmol/L lower after gliclazide therapy (Table 2); they were lower in 14 of the 17 periods. The lower plateau glucoses were not related to higher plateau plasma insulin concentrations. In fact, in 10 of these 14 periods, the steady-state plasma insulin was also lower.

DISCUSSION

Our results indicate that the chronic hypoglycemic action of gliclazide in obese diabetic subjects is associated with improvement in insulin action. It is not associated with an increase in erythrocyte or monocyte insulin receptor binding, in contrast to findings reported after therapy with chlorpropamide[6] and glibenclamide.[16] The absence of an effect on receptor binding and the observed increase in insulin action are consistent with the potentiation of pathways distal to insulin binding.

The trend to a decrease in insulin binding may have been related to the gain in weight that occurred in all except one subject (whose receptor binding increased). The subjects

were instructed not to alter their food intake during the study and some of the weight gain might be attributed to a decrease in glycosuria and energy loss. Although the effect of placebo was not tested in this study, gliclazide has been shown to have a specific efficacy over and above placebo.[17] A double-blind, crossover study was not performed because we felt chronic treatment with a placebo may not be completely safe in this group, which had moderately severe hyperglycemia and in whom dietary therapy had failed. In addition, it was considered likely that such a study would not be really blind in these particular circumstances, as the lack of response to placebo would be obvious. The fact that the subjects gained weight yet exhibited an improvement in insulin-stimulated glucose utilization supports an effect related to the gliclazide rather than the response being due simply to stricter dietary adherence. Furthermore, it should be noted that, before entering the gliclazide study, the subjects had already had a trial of dietary therapy alone without achieving adequate glycemic control. Therefore, it appears that the improvement in insulin action cannot be attributed to improved dietary compliance, which is the main variable that would be controlled by a randomized crossover study.

The mechanism of the postreceptor effect of gliclazide therapy cannot be deduced from this study. Other studies have shown that treatment with insulin may partially reverse the insulin resistance[18] and postreceptor defect[19] in non-insulin-dependent diabetic subjects. This raises the possibility that improvement in insulin action could be secondary to improved insulin secretion or improved metabolic control per se. On the other hand, recent studies in vitro show a direct effect of sulfonylurea drugs to increase postreceptor insulin action.[8,9] The effect of gliclazide in vivo on postbinding pathways might, therefore, be separate from any effect secondary to improved insulin secretion or control of glycemia.

We were not able to demonstrate a significant change in R_d or MCR_g at the lowest insulin infusion rate. This was unexpected, given the significant increases in both R_d and MCR_g at the higher levels of infused insulin. It is possible that gliclazide had no effect on insulin action at the lowest insulin level. On the other hand, gliclazide might have had an effect at low levels of plasma insulin, masked in some subjects by their decrease in insulin receptor binding. Furthermore, increases in R_d and MCR_g at the low insulin infusion rate may not have been detected due to methodologic limitations. For example, we would expect a change in insulin responsiveness to produce smaller, less easily detected, changes in MCR_g at the lowest level than at the higher levels.

In our study, insulin-mediated glucose utilization was shown to be increased at the two higher insulin infusion rates whether R_d or MCR_g were used. We have included MCR_g in our report because the plateau plasma glucose concentrations were slightly lower (mean 0.4 mmol/L) in the studies after gliclazide therapy. MCR_g provides a reasonable correction for these small differences, because it has been shown that, if insulin levels are above 25 mU/L and glucose concentrations are less than 11 mmol/L, MCR_g is independent of the glucose concentration while R_d varies.[14,20] As can be seen from Table 2, the lower plateau glucose levels after gliclazide therapy were not caused by higher plateau insulin levels, because in 10 of these 14 periods plateau insulins were in fact lower. This is probably a reflection of the improvement in insulin action. Nevertheless, the slightly lower plateau glucose levels after gliclazide therapy could introduce a small bias to the measurement of R_d. To achieve identical plateau glucoses would have necessitated a higher glucose infusion rate. This would have caused the R_d values to have been even higher in the posttherapy periods. We would, therefore, expect the small difference in plateau glucoses to cause a relative underestimation of the posttherapy R_d values, which could not, therefore, account for the increases in R_d that we observed after therapy.

In addition to the improvement in insulin action, our results would be consistent with an improvement in β-cell function after gliclazide therapy. For example, although basal insulin increased insignificantly from 25 to 31 mU/L, the basal glucose fell from 13.4 to 8.4 mmol/L, which suggests that the β-cells secreted at least as much insulin despite lower blood glucose levels. In addition, there was a trend to an increase in the incremental insulin area after OGTT despite reduced glycemic stimulus (reflected by the decreased glucose areas).

Our findings contrast with those reported recently by Marchand et al.,[21] who were unable to demonstrate an effect of gliclazide on insulin action in vivo. This lack of accord may be due to one or more differences in experimental design or subject selection. Marchand et al.[21] used a less-sensitive euglycemic clamp technique involving bolus doses of insulin, and their subjects were near-normal in weight and had milder diabetes. Our data showed an improvement in insulin action at high but not low plasma insulin levels, associated with chronic gliclazide treatment in obese diabetic subjects. This enhanced insulin action was not accompanied by an increase in binding to erythrocyte or monocyte insulin receptors, suggesting potentiation of postbinding pathways of insulin action.

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