Insulin Receptor Binding to Monocytes, Insulin Secretion, and Glucose Tolerance Following Metformin Treatment

Results of a Double-Blind Cross-Over Study in Type II Diabetics

R. PRAGER AND G. SCHERNTHANER

SUMMARY

We studied the effect of metformin therapy (1700 mg daily) on glucose tolerance, insulin secretion, and insulin binding to monocytes in 10 non-insulin-dependent diabetics (mean duration of disease 2.6 yr) who were treated for 4 wk with either metformin or placebo in a double-blind cross-over study.

Metformin induced a significant decrease of glucose levels during an oral glucose load compared with placebo treatment ($P < 0.001$). All patients studied showed normal or elevated basal insulin and C-peptide levels; their responses to an oral glucose load, however, were relatively hypoinsulinemic without any significant difference between metformin and placebo. Insulin binding to monocytes was nearly identical at all insulin concentrations tested in the placebo or metformin therapy phase.

These data indicate that the glucose-lowering potency of metformin in non-insulin-dependent diabetics cannot be associated with changes in receptor number or affinity. It is suggested that metformin might have a positive influence on postreceptor defects in non-insulin-dependent diabetics. DIABETES *32*:1083–1086, December 1983.

Biguanides as well as sulfonylureas have proved to be an effective treatment of non-insulin-dependent diabetes mellitus. After discontinuation of biguanides deterioration of metabolic control was observed in a large number of non-insulin-dependent diabetics.[1] The mechanism of the hypoglycemic action of biguanides still remains speculative. The three major hypotheses concerning their action involve facilitation of cellular oxidation of glucose,[2] inhibition of hepatic gluconeogenesis,[3] and impairment of glucose absorption through the gastrointestinal tract.[4,5] Since biguanides as well as sulfonylureas are most effective in diabetics who can secrete insulin, it has been speculated that both drugs may potentiate the effects of insulin at the cellular level. The first step in insulin action is binding to its target cell receptor. Sulfonylureas are thought to have a positive influence on insulin receptor binding,[6–9] and there is some evidence that biguanides may enhance insulin receptor binding in vivo and in vitro[10–12] similarly. To test whether receptor effects are induced by metformin treatment in non-insulin-dependent diabetics and whether such mechanisms are of clinical significance regarding the hypoglycemic action of biguanides as recently suggested,[12] glucose tolerance and insulin receptor binding to monocytes were investigated in non-insulin-dependent diabetics treated with either metformin or placebo in a double-blind cross-over design.

From the Department of Medicine II, University of Vienna, and Ludwig Boltzmann-Institute for Clinical Endocrinology, Vienna, Austria.
Address reprint requests to Univ. Doz. Dr. G. Schernthaner, Department of Medicine II, Garnisongasse 13, A-1090 Vienna, Austria.
Received for publication 13 January 1983 and in revised form 9 May 1983.

METHODS

Subjects. The effect of metformin treatment on insulin binding to monocytes, glucose tolerance, and insulin secretion was investigated in 12 obese non-insulin-dependent diabetics according to the WHO criteria.[13] These patients were treated with diet alone and were without a history of any oral hypoglycemic medication.

To examine the effect of metformin treatment, a double-blind cross-over study design was used. Six patients received 2 × 850 mg metformin per day, and six patients were treated with placebo for a period of 2 wk. The treatment was then reversed to placebo or metformin, respectively, for another 2 wk. Drug adherence was estimated by measurement of metformin plasma levels,[14] and two patients (1 male, 1 female) were excluded from the study since plasma levels of metformin were not detectable. The clinical data of the remaining 10 patients, including age, duration of disease, height, weight, fasting blood glucose, glycosylated hemoglobin, as well as basal immunoreactive insulin and C-peptide, are summarized in Table 1. All patients studied were free from vascular complications and presented with normal hepatic and renal functions.

TABLE 1
Clinical data and laboratory parameters of the patients studied (mean ± SEM)

Patients				Duration	Body		Fasting	Glycosylated	Immunoreactive	Immunoreactive
No.	Initials	Sex	Age (yr)	of disease (yr)	weight (kg)	Height (cm)	blood glucose (mg/dl)	hemoglobin (%)	insulin (μU/ml)	C-peptide (ng/ml)
1	B.H.	F	54	4	79	150	154	13.6	11.4	2.27
2	L.K.	F	68	1	75	160	296	12.6	12.8	2.72
3	R.K.	M	55	0.5	150	175	125	7.6	12.6	2.82
4	O.A.	F	58	6	100	163	153	8.8	16.0	2.76
5	M.A.	F	50	3	75	168	123	8.2	9.4	2.07
6	K.J.	M	59	2	88	174	266	11.1	21.9	3.25
7	E.J.	M	41	2	95	172	126	7.6	24.6	2.97
8	R.A.	F	60	8	63	153	156	9.8	14.8	6.69
9	U.A.	F	57	1	92	157	230	11.1	18.9	3.53
10	S.B.	F	62	1	95	172	142	10.0	10.6	3.6

Insulin binding to monocytes and an oral glucose tolerance test (50 g glucose) with measurement of blood glucose levels and immunoreactive insulin and C-peptide concentrations were performed after 2 wk at the time of medication change and after 4 wk at the end of the study. Glucose was determined by the glucose-oxidase method, immunoreactive insulin and C-peptide by radioimmunoassays.

HbA_1 was measured by microcolumn chromatography after a 24-h dialysis of the hemolysates.

Cell binding studies. Insulin binding studies were carried out in the morning following an overnight fast. Blood (120 ml) was drawn into tubes containing EDTA (dipotassium salt). Mononuclear leukocytes were isolated by gradient centrifugation.[15] Insulin binding studies to mononuclear leukocytes were performed according to the method of Beck-Nielsen et al.[16] The cells were washed twice and incubated in a Hepes buffer (100 mmol/L, pH 7.8 at 15°C) at a concentration of approximately 5×10^7/ml for 100 min with ^{125}I TYR A14 insulin at a concentration of 34 pmol/L (0.2 ng/ml). The specific activity of the tracer was about 223 μCi/μg. Native insulin was added in increasing amounts to the incubation medium for the competition studies. Cell bound and free insulin were separated by centrifugation after the incubation period. The specific cell binding fraction was defined as total cell binding fraction minus nonspecific cell binding, i.e., radioactivity that remained bound in the presence of an excess of native insulin (10 μmol). This fraction averaged approximately 8% of the total binding. Monocytes were identified by alpha-naphthylacetyl-esterase-staining[17] and specific cell binding fraction was adjusted to a standard concentration of monocytes of 1.0×10^7 ml, using the formula described by Beck-Nielsen et al.[16]

Cell binding analysis. The results of the binding study are presented in three ways: the specific cell bound fraction is plotted as a function of the total insulin concentration (competition curve), the bound free insulin ratio is plotted as a function of bound insulin (Scatchard plot),[18] and the average affinity has been calculated according to the method of De Meyts.[19] In this study we have defined the highest binding affinity (KE) as the value of binding affinity, as the insulin tracer concentrations of 34 pmol/L.

Statistics. For comparison of all data obtained during the metformin and placebo phase, Student's *t* tests for paired data were used as appropriate. Data from both arms of the placebo and metformin groups were combined for the analysis, since we observed no significant variability of the placebo and metformin data during the separate arms of the study.

RESULTS

During metformin treatment no significant change in body weight was observed. Serum creatinine and parameters of liver function also remained unchanged. Metformin was well tolerated and in none of the patients side effects necessitated discontinuation of treatment.

Compared with placebo administration, metformin caused a significant lowering of blood glucose levels after an oral glucose load (Figure 1). Serum insulin and C-peptide levels during oral glucose tolerance test were not significantly influenced by metformin compared with placebo (Figure 1). Insulin receptor binding was nearly identical during the metformin period compared with the placebo treatment period. Neither absolute receptor number nor absolute receptor affinity was significantly changed by metformin (Figure 2). The binding affinity at insulin tracer concentrations was 3.74×10^{-8} mol/L in the placebo phase and 3.89×10^{-8} mol/L in the metformin period.

DISCUSSION

In the present study, the hypoglycemic action of metformin was confirmed in non-insulin-dependent diabetics.[20] In accordance with previous reports the improvement of glucose tolerance was not paralleled by changes in insulin secretion.[21,22] However, the fact that insulin levels are not changed on an absolute basis may suggest that insulin secretion is relatively enhanced since glucose levels were lower after metformin therapy. In our study, the glucose-lowering potency of metformin could not be related to changes in absolute receptor number or affinity. This contrasts with recent in vitro data.[10,11] In the latter studies, an increase in insulin receptor binding following biguanide administration was observed in different cell types, which was related to an increase in insulin receptor affinity. Besides these in vitro data, a positive in vivo effect on insulin receptors of healthy volunteers was recently reported,[12] although this effect was rather small compared with in vitro enhancement of insulin binding. The apparent discrepancy may reflect the fact that erythrocytes were used as target cells in a study of Holle et

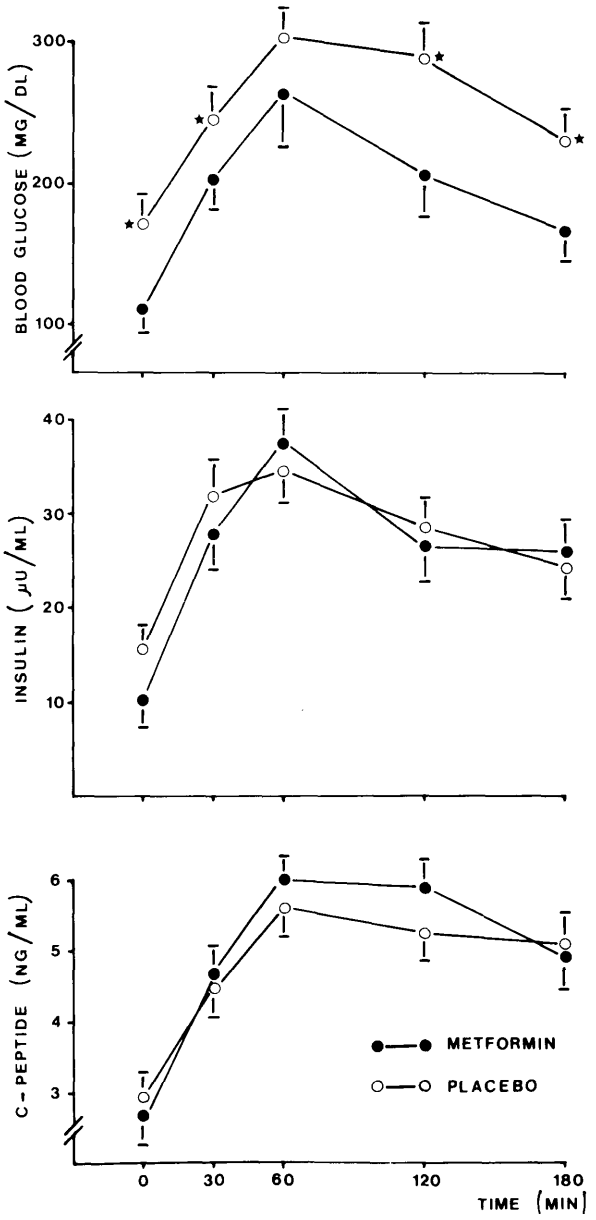

FIGURE 1. Blood glucose, immunoreactive insulin, and immunoreactive C-peptide after an oral glucose load (mean ± SEM). *P < 0.001.

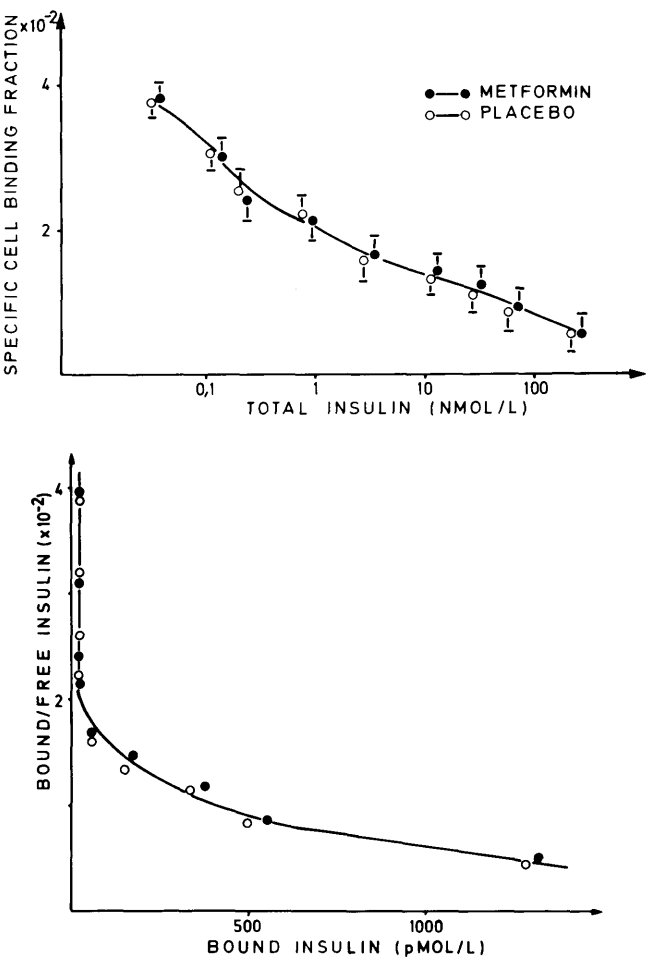

FIGURE 2. Specific insulin binding to monocytes after placebo and metformin treatment and Scatchard analysis of the binding data.

al.[12] This cell type has lost the capacity for protein de novo synthesis and therefore for receptor upregulation in the cell membrane. The limitations of insulin receptor determinations on erythrocytes have been previously demonstrated.[23–25]

Besides the inhibitory effect of biguanides on hepatic gluconeogenesis[3] and intestinal glucose absorption,[4,5] there is some evidence that peripheral insulin resistance might be improved after biguanide administration. Increased cellular glucose uptake and increased glucose extraction from the muscle after biguanides support a peripheral action of these compounds.[26–29] Since insulin receptor behavior remained completely unchanged after therapy, a possible positive effect of biguanides on peripheral insulin action must be located distal to the initial binding step. Furthermore, postreceptor defects seem to be the cause of peripheral insulin resistance in non-insulin-dependent diabetics[30–32] and changes in insulin receptor number or affinity would have little if any influence on glucose tolerance improvement. In this connection, preliminary data of Tiengo et al.[33] are of some interest. This group demonstrated (using the euglycemic clamp technique) an increased glucose utilization after metformin therapy without detectable changes in insulin sensitivity.

We conclude that the hypoglycemic action of metformin in non-insulin-dependent diabetics with a relatively low insulin response does not seem to be related to effects on receptor levels as concluded from in vitro data.[10,11] A possible positive effect of metformin on peripheral insulin action can only be due to an improvement of postreceptor defects.

ACKNOWLEDGMENTS

We thank Dr. Renate Mathae for her helpful medical assistance and Wilhelm Temsch for his excellent technical assistance.

REFERENCES

[1] Siitonen, O., Aro, A., Huttunen, J. K., Juvonen, H., Järvinen, R., Palomäki, P., and Ritala, P.: Effect of discontinuation of biguanide therapy on metabolic control in maturity onset diabetes. Lancet 1980; 1:217–20.

[2] Butterfield, W. J. H., and Whichelow, M. J.: The hypoglycemic action

of phenformin. Effect of phenformin on glucose metabolism in peripheral tissues. Diabetes 1962; 213:11:281–86.

[3] Meyer, F., Ipacktchi, M., and Clauser, H.: Specific inhibition of gluconeogenesis by biguanides. Nature 1967; 213:203–204.

[4] Czyzyk, A., Tawecki, J., Sadowski, J., Ponikowska, I., and Szczepanik, Z.: Effect of biguanides on intestinal absorption of glucose. Diabetes 1968; 17:482–88.

[5] Lorch, E.: Inhibition of intestinal absorption and improvement of glucose tolerance by biguanides in the normal and in the streptozotocin-diabetic rat. Diabetologia 1971; 7:195–203.

[6] Olefsky, J. M., and Reaven, G. M.: Effects of sulfonylureas therapy on insulin binding to mononuclear leucocytes of diabetic patients. Am. J. Med. 1976; 60:89–95.

[7] Beck-Nielsen, H., Pedersen, O., and Linskov, H. O.: Increased insulin sensitivity and cellular binding in obese diabetics following treatment with glibenclamide. Acta Endocrinol. (Kbh) 1979; 90:451–62.

[8] Feinglos, M. N., and Lejbovitz, H. E.: Sulfonylureas increase the number of insulin receptors. Nature 1978; 276:184–85.

[9] Prince, M. J., and Olefsky, J.: Direct in vitro effect of a sulfonylurea to increase human fibroblast insulin receptors. J. Clin. Invest. 1980; 66:608–11.

[10] Cohen, D., Pezzino, V., Vigneri, R., Avola, R., D'Agata, F., and Polosa, P.: Phenformin increases insulin binding to human cultured breast cancer cells. Diabetes 1980; 29:329–31.

[11] Vigneri, R., Pezzino, V., Wong, K. Y., and Goldgine, I. D.: Comparison of the in vitro effect of biguanides and sulfonylureas on insulin binding to its receptors in target cells. J. Clin. Endocrinol. Metab. 1982; 54:95–100.

[12] Holle, A., Mangels, W., Dreyer, M., Kühnau, J., and Rüdiger, H. W.: Biguanide treatment increases the number of insulin receptor sites on human erythrocytes. N. Engl. J. Med. 1981; 305:563–66.

[13] WHO-Expert Committee on Diabetes Mellitus 1980; WHO-Tech. Rep. 646.

[14] Brohon, J., and Noel, M.: Determination of metformin in plasma at therapeutic levels by gas-liquid chromatography using a nitrogen detector. J. Chromatogr. 1978; 146:148–51.

[15] Boyum, A.: Separation of leucocytes on blood and bone marrow. Scand. J. Lab. Clin. Invest. 1968; 22 (Suppl. 97):77–89.

[16] Beck-Nielsen, H., Pedersen, O., Kragballe, K., and Sörensen, N. S.: The monocyte as a model for the study on insulin receptors in men. Diabetologia 1977; 13:563–69.

[17] Yam, L. T., Li, C. Y., and Crosby, W. N.: Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol. 1971; 55:283–86.

[18] Scatchard, G.: The attraction proteins for small molecules and ions. Ann. NY Acad. Sci. 1949; 51:660–72.

[19] De Meyts, P., and Roth, J.: Cooperativity in ligand binding: a new graphic analysis. Biochem. Biophys. Res. Commun. 1975; 66:1118–26.

[20] Sterne, J.: Pharmacology and mode of action of the hypoglycemic guanidine derivates. *In* Oral Hypoglycemic Agents: Pharmacology and Therapeutics. Campbell, G. D., Ed. New York, Academic Press, 1969:133–245.

[21] Schatz, H., Katsilambros, N., Nierle, C., and Pfeiffer, E. F.: The effect of biguanides on secretion and biosynthesis of insulin in isolated pancreatic islets of rats. Diabetologia 1972; 8:402–407.

[22] Carpentier, J. L., Luyckx, A. S., and Lefebvre, P. J.: Influence of metformin on arginine-induced glucagon secretion in human diabetes. Diabete Metab. 1975; 1:22–28.

[23] De Pirro, R., Fusco, A., Lauro, R., Testa, I., Ferreti, G., and Demartinis, C.: Insulin receptors on monocytes and erythrocytes from obese patients. J. Clin. Endocrinol. Metab. 1980; 51:1437–39.

[24] Ward, G. M., Rees, A. R., Naylor, B. A., and Turner, R. C.: The relationship of erythrocyte insulin receptors to red cell age and to monocyte insulin receptors. Clin. Endocrinol. 1981; 14:269–78.

[25] Prager, R., and Schernthaner, G.: Comparison of insulin receptor binding to monocytes and erythrocytes in newly diagnosed type-II diabetes mellitus. Horm. Metab. Res. 1982; 14:561–63.

[26] Butterfield, W. H. J., and Whichelow, M. J.: Effect of diet, sulphonylureas and phenformin on peripheral glucose uptake in diabetes and obesity. Lancet 1968; 2:785–88.

[27] Frayn, K. N.: Effects of metformin on insulin resistance after injury in the rat. Diabetologia 1976; 12:53–60.

[28] Wicklmayr, M., Dietze, G., and Mehnert, H.: Effect of phenformin on substrate metabolism of working muscle in maturity onset diabetes. Diabetologia 1979; 15:99–104.

[29] Frayn, K. N., and Adnitt, P. I.: Effects of metformin on glucose uptake by isolated diaphragm from normal and diabetic rats. Biochem. Pharmacol. 1972; 21:3153–62.

[30] Olefsky, J. M.: Insulin resistance and insulin action. An in vitro and in vivo perspective. Diabetes 1981; 30:140–62.

[31] Kolterman, O. G., Gray, R. S., Griffin, J., Burstein, P., Insel, J., Scarlett, J. A., and Olefsky, J. M.: Receptor and post-receptor defects contribute to insulin resistance in non-insulin dependent diabetes mellitus. J. Clin. Invest. 1981; 68:957–69.

[32] Scarlett, J. A., Gray, R. S., Griffin, J., Olefsky, J. M., and Kolterman, O. G.: Insulin treatment reverses the insulin resistance of type-II diabetes mellitus. Diabetes Care 1982; 5:353–63.

[33] Tiengo, A., Del Prato, S., Padovan, D., Nosadini, R., Duner, E., Marescotti, C., Avogaro, A., Trevisan, R., and Sigili de Kreuzenberg, S.: Improvement of post-receptor insulin defect in type-2 (non-insulin-dependent) diabetic patients by metformin. Abstract. Diabetologia 1982; 23:205.