New α_2-Adrenergic Blocker (DG-5128) Improves Insulin Secretion and In Vivo Glucose Disposal in NIDDM Patients

A. KASHIWAGI, Y. HARANO, M. SUZUKI, H. KOJIMA, M. HARADA, Y. NISHIO, AND Y. SHIGETA

SUMMARY

Effects of oral administration of DG-5128, a new oral hypoglycemic agent, on glycemic control after a mixed meal and an in vivo glucose disposal were measured in subjects with nonobese non-insulin-dependent diabetes mellitus (NIDDM). Oral administration of DG-5128 significantly ($P < .05$) enhanced insulin secretion both 30 and 60 min after a mixed meal (550 kcal), with a concomitant decrease in postprandial plasma glucose levels at 60 and 120 min. Glucose disposal rate between the 2nd and 4th h of a euglycemic insulin clamp, developed through a constant infusion of insulin (0.77 mU · kg^{-1} · min^{-1}) together with somatostatin (80 ng · kg^{-1} · min^{-1}), was 2.5-fold higher in a DG-5128–treated group ($P < .01$) than in a control group. However, there was no difference between the two groups in either plasma glucose concentration or plasma insulin concentration at either the 2nd or the 4th h. These results indicate that DG-5128 is effective in controlling plasma glucose levels in subjects with NIDDM by stimulation of both insulin secretion and in vivo glucose disposal. DIABETES 1986; 35:1085–89.

The new orally effective hypoglycemic agent 2-[2-(4,5-dihydro-1*H*-imidazol-2-yl)-1-phenylethyl] pyridine dihydrochloride sesquihydrate (DG-5128) has been recently developed by Kameda et al.[1] DG-5128 has been found to decrease blood glucose levels in normal and diabetic animals[1] and in healthy humans[2] by stimulating insulin release.[3,4] Interestingly, it has been shown that DG-5128 significantly reverses an α_2-adrenergic inhibition of the glucose-primed insulin release from the islets.[3]

We describe the possible effectiveness of DG-5128 for diabetic control in subjects with NIDDM through the stimulation of insulin secretion after a mixed meal and in vivo glucose disposal as measured by the euglycemic insulin clamp.

From the Third Department of Medicine, Shiga University of Medical Science, Seta, Ohtsu, Japan 520-21.
Address reprint requests to A. Kashiwagi, M.D., at the above address.
Received for publication 18 December 1985 and in revised form 18 April 1986.

MATERIALS AND METHODS

Nine subjects with NIDDM who were admitted to the University Hospital, Shiga University of Medical Science, and given a diet (27–30 kcal/kg ideal body wt) consisting of 18% protein, 25% fat, and 57% carbohydrate for at least 10 days were investigated. Diabetes mellitus was diagnosed with the criteria: fasting plasma glucose ≥140 mg/dl and/or 2 h PG ≥ 200 mg/dl after a 75-g oral glucose tolerance test. All subjects were relatively mild diabetics and had been treated only with diet. Informed consent was obtained from all the subjects. Characteristics of the subjects are summarized in Table 1.

MEASUREMENTS

Glucose-clamp studies. Glucose-clamp studies were performed after an overnight fast as described previously.[5] Human monocomponent insulin (Novo, Copenhagen, Denmark) was administered as a primed continuous infusion (5.8 mU/kg body wt i.v.) over the first 5 min followed by a constant infusion of insulin (0.77 mU · kg^{-1} body wt · min^{-1}) together with somatostatin (80 ng · kg^{-1} body wt · min^{-1}) with a constant-infusion pump (Nikkiso, Kyoto, Japan). A heparinized blood sample was continuously obtained through an indwelling intravenous silicone double-lumen catheter placed in the antecubital vein, and blood glucose concentration was continuously monitored by a glucose monitor with a glucose oxidase method (Kyoto Daiichi Kagaku, Kyoto, Japan). A glucose solution (12%) containing 5 meq/L KCl was administered by an infusion pump (TFV-1100, Nihon Kohden, Tokyo, Japan) at rates based on a negative-feedback algorithm. The algorithm was aimed at maintaining blood glucose concentration at the fasting level if fasting blood glucose level was <100 mg/dl and at 100 mg/dl if fasting blood glucose

TABLE 1
Characteristics of subjects

Group	Subject	Age (yr)	Sex	BMI (kg/m²)	FPG (mg/dl)	HbA_1 (%)	IRI (μU/ml)
Control	M.N.	53	F	20.8	122	7.5	12
	Z.S.	49	M	20.6	129	8.4	11
	S.K.	68	F	22.8	132	9.2	12
	K.F.	53	F	21.1	126	11.3	12
Mean ± SE		56 ± 4		21.3 ± 0.4	127 ± 2	9.1 ± 0.7	12 ± 0.3
DG-5128	S.N.	47	F	23.8	89	7.8	11
	N.O.	60	M	20.7	120	7.6	15
	F.K.	50	M	18.7	118	7.2	11
	O.T.	58	F	20.4	86	7.7	9
	M.K.	54	M	20.2	105	11.0	9
Mean ± SE		54 ± 2		20.8 ± 0.7	104 ± 6	8.3 ± 0.6	11 ± 2

BMI, body mass index; FPG, fasting plasma glucose; HbA_1, hemoglobin A_1; IRI, immunoreactive insulin.

level was >100 mg/dl at the start of infusion. Glucose infusion rate was calculated by the formula:

$$[GI(t) = k_1/100[TG\text{-}PBG(t)] - 1/2(k_2/100)(-dG(t)/dt) + k_3/a\int_{t-a}^{t} G(t)dt$$

where k_1 is a constant value, TG is target glucose concentration, PBG is predicted blood glucose concentration (mg/dl) 4 min after the time (t); k_2 is a constant value, $dG(t)/dt$ is a slope of blood glucose concentration (mg/2 min) calculated from approximation of seven previous serial measurements; and k_3 is a constant value, $1/a \int_{t-a}^{t} G(t)dt$ is mean value of glucose infused before t. For the routine tests, empirically obtained optimum values (k_1 = 5, k_2 = 12, and k_3 = 100) were used. Coefficient of variation of monitored blood glucose was within 5% between 1 and 2 h and between 3 and 4 h. The mean blood glucose values were also well fixed at the aimed (clamped) glucose level. Occasional manual changes of constant values were required for a strict clamp of blood glucose level. Glucose loss in the urine was negligible in our study. For the measurement of hepatic glucose output, [^{3}H]glucose was not acceptable for use in human subjects. Because hepatic glucose output in nonobese and obese diabetic subjects was suppressed to 7% of the preinfusion level during the hyperinsulinemic clamp,[6] the glucose infusion rate was substituted for an estimation of glucose disposal rate.

Standard-meal test. Four subjects with NIDDM were studied on two separate days. On the first day, 550 kcal of the standard mixed meal (60% carbohydrate, 14% fat, and 26% protein) was given. Blood samples were obtained from an antecubital vein 0, 30, 60, 120, and 180 min after the start of the test meal. On the following day, 200 mg of DG-5128 was orally administered 30 min before the start of the same test meal. Blood samples were obtained at the same time schedule as control experiments.

Plasma glucose concentrations were determined by the hexokinase method.[7] Plasma insulin concentrations were measured as previously described[8] with the RIA kit from Midorijuji Radioisotope Laboratory, and the RIA kit from Shionogi Pharmaceutical was used for the measurement of plasma C peptide immunoreactivity.[9] Hemoglobin A_1 was determined by the method of Trivelli et al.[10]

Statistical methods. The two-tailed Student's t test was used to analyze the significant differences. Either the paired or nonpaired t test was used for evaluation of the difference between two samples. All data are expressed as means ± SE.

RESULTS

Effects of DG-5128 on postprandial glycemic control. As shown in Fig. 1*A*, oral administration of 200 mg of DG-5128 30 min before the start of a test meal (DG-5128–treated group) significantly ($P < .01$) decreased plasma glucose

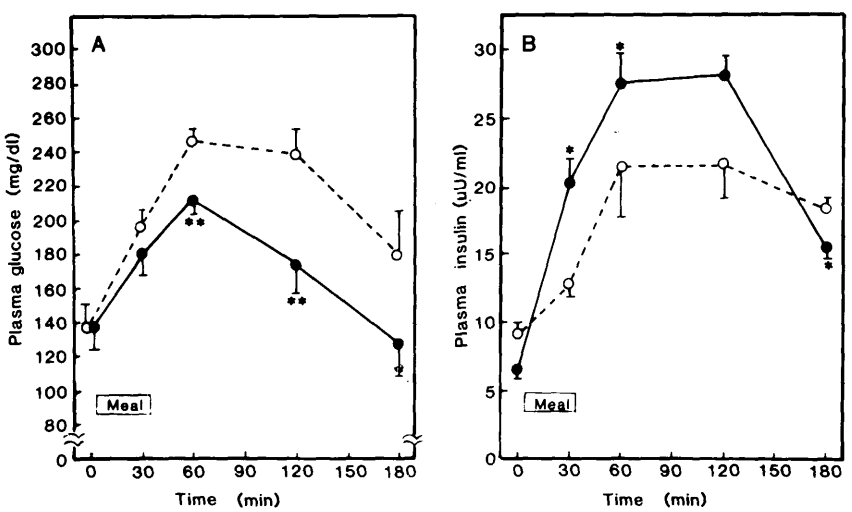

FIG. 1. Changes in plasma glucose (*A*) and plasma insulin (*B*) concentration after intake of mixed meal (550 kcal). Four subjects (S.K., K.F., O.T., and M.K., as described in Table 1) were given placebo tablets (○) and 200 mg of DG-5128 (●) on serial days, respectively, 30 min before intake of mixed meal. Data are expressed as means ± SE (N = 4). *$P < .05$, **$P < .01$ (by paired t test).

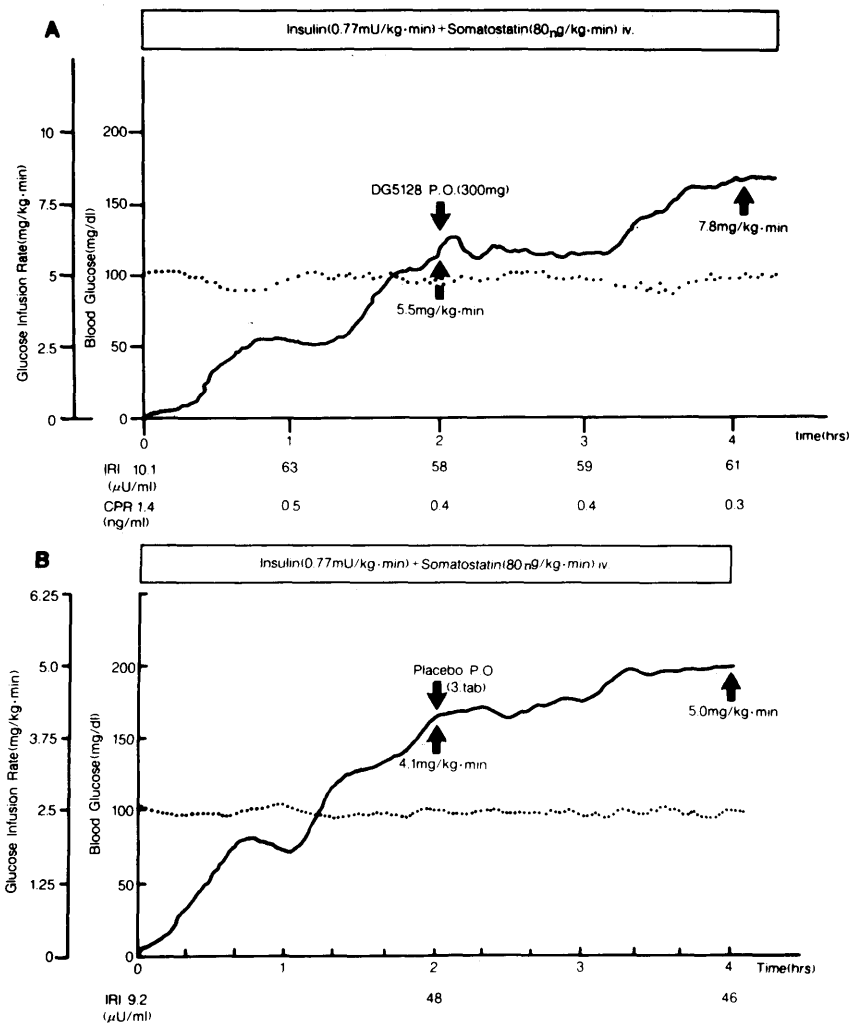

FIG. 2. Four-hour euglycemic insulin clamp in DG-5128–treated subject (*A*) and placebo-treated subject (*B*). *Solid line* depicts glucose infusion rate (mg · kg^{-1} · min^{-1}), and *dotted line* indicates blood glucose concentration measured every 2 min. Either placebo or DG-5128 (300 mg) is orally administered at 2 h of euglycemic insulin clamp. Glucose infusion rates at 2nd and at 4th h of insulin clamps are shown with *arrows*. By infusing 0.77 mU · kg^{-1} · min^{-1} insulin and 80 ng · kg^{-1} · min^{-1} somatostatin, constant higher levels of plasma IRI and constant lower levels of plasma C peptide are kept during last 2 h of clamp study, compared with preinfusion level, respectively, as shown on *bottom* of *A* and *B*.

levels at 60 and 120 min after an intake of a mixed meal, compared with the same subjects who had taken only the test meal the day before (a control group). In contrast, plasma immunoreactive insulin (IRI) levels at 30 min (21 ± 2 μU/ml) and 60 min (27.5 ± 3 μU/ml) in the DG-5128–treated group were significantly ($P < .05$) higher than those at 30 min (12.8 ± 0.8 μU/ml) and 60 min (21.4 ± 4 μU/ml) in the control group. On the other hand, plasma IRI concentration at 180 min (8.3 ± 0.5 μU/ml) in the DG-5128–treated group was significantly ($P < .05$) lower than that in the control group (15.5 ± 0.8 μU/ml; Fig. 1*B*).

Effects of DG-5128 on in vivo glucose disposal. Typical results with the euglycemic insulin clamp in a control and a DG-5128–treated subject are depicted in Fig. 2, *A* and *B*, respectively. By infusing insulin at the rate of 0.77 mU · kg^{-1} · min^{-1} together with somatostatin at the rate of 80 ng · kg^{-1} · min^{-1}, basal plasma IRI (10.1 μU/ml) was raised to 63 μU/ml 1 h after an initiation of infusion. A 300-mg oral dose of DG-5128 enhanced glucose disposal rate at the 4th h by 42% above the initial steady-state level (at 2 h) without any significant changes in plasma IRI level during the last 2 h. During the entire experiment, endogenous insulin secretion as measured by C peptide concentration was suppressed by >64% (from 1.4 to 0.5 ng/ml; Fig. 2*A*).

In contrast, the placebo group exhibited a 22% natural increase in glucose disposal by the 4th h over the 2nd h (Fig. 2*B*). The increase in glucose disposal rate between the 2nd and 4th h of the glucose clamp in the DG-5128–treated group (2.8 ± 0.3 mg · kg^{-1} · min^{-1}) was significantly higher ($P < .01$) than that in the control group (1.1 ± 0.3 mg · kg^{-1} · min^{-1}; Table 2). Furthermore, stimulation of glucose disposal rate at the 4th h in the DG-5128–treated group was increased by 66 ± 9% above the value at the 2nd h, which was significantly higher ($P < .01$) than that in the control group (23 ± 5%). On the other hand, steady-state plasma glucose and insulin levels were not significantly different between the 2nd and 4th h in the control and the DG-5128–treated group. There was no difference in steady-state plasma glucose and insulin levels for the last 2 h between the control and DG-5128–treated groups.

DISCUSSION

Our study demonstrates that oral administration of the new oral hypoglycemic agent DG-5128 significantly decreases hyperglycemia after intake of a mixed meal in subjects with NIDDM. Previously, DG-5128 was shown to be effective in reducing postprandial blood glucose levels in normal and diabetic animals[1] as well as in healthy human subjects,[2] based on the stimulation of insulin release through an an-

TABLE 2
Effect of DG-5128 on in vivo glucose disposal in subjects with NIDDM

		Steady-state level				Glucose disposal rate			
		PG (mg/dl)		IRI (μU/ml)		mg · kg^{-1} · min^{-1}			%
Group	Subject	B	A	B	A	B	A	A − B	100(A − B)/B
Control	M.N.	81	83	54	55	4.3	4.6	0.3	6
	Z.S.	102	100	48	46	4.1	5.0	0.9	22
	S.K.	100	99	52	50	5.0	6.5	1.5	30
	K.F.	106	91	52	52	5.5	7.3	1.8	33
Mean ± SE		97 ± 5	93 ± 3	52 ± 1	51 ± 2	4.8 ± 0.2	5.9 ± 0.6	1.1 ± 0.3	23 ± 5
DG-5128	S.N.	73	76	38	48	3.8	5.5	1.7	45
	N.O.	99	100	30	32	4.5	8.5	4.0	88
	F.K.	92	97	58	61	5.5	7.8	2.3	42
	O.T.	89	84	63	57	3.6	6.0	2.4	67
	M.K.	112	116	46	37	3.9	7.3	3.4	87
Mean ± SE		93 ± 6	95 ± 7	47 ± 5	47 ± 5	4.3 ± 0.3	7.0 ± 0.5	2.8 ± 0.3*	66 ± 9*

B, value at 2nd h; A, value at 4th h of glucose clamp.
*$P < .01$ (nonpaired t test, compared with that of control group).

tagonizing effect at α_2-adrenergic receptors on the β-cells.[3,4] In our study, DG-5128 also significantly enhanced insulin secretion 30 and 60 min after an intake of a test meal in subjects with NIDDM. These data indicate that DG-5128 may be useful for the control of postprandial blood glucose concentration in subjects with NIDDM.

Our study also demonstrates a peripheral enhancement of in vivo glucose disposal rate. The stimulation of glucose disposal by orally administered DG-5128 was quantified by an increase in glucose disposal rate between the 2nd and 4th h of the euglycemic insulin clamp. As described previously, in vivo glucose utilization gradually increased during a prolonged glucose clamp.[11] In our study, we carefully matched age, degree of obesity, and severity of diabetic state of subjects between a control and a DG-5128–treated group because the magnitude of a gradual increase in glucose disposal might be affected by those factors.[11] A mean increase in glucose disposal rate during the last 2 h of the insulin clamp in control subjects with NIDDM was only 23%. The value was smaller than that previously reported.[11] A smaller increase in glucose disposal rate during the last 2 h of the insulin clamp in our control diabetic subjects might be due to a lower steady-state plasma insulin level than that reported by Doberne et al.[11] Orally administered DG-5128 enhanced in vivo glucose disposal 2.5-fold compared with that of a control group. No significant difference in plasma IRI levels between the 2nd and 4th h in either control or DG-5128–treated subjects was observed because of the presence of somatostatin. Therefore it is unlikely that the DG-5128–dependent stimulation of glucose disposal during the euglycemic insulin clamp was mediated by the stimulation of insulin release. Note also that our euglycemic insulin-clamp study was carried out by obtaining blood samples for measuring glucose concentrations from an antecubital vein, which contained mixed venous blood, and not from an arterialized hand vein. Therefore, if in vivo peripheral glucose utilization was stimulated by oral administration of DG-5128, to maintain constant plasma glucose level, arterial hyperglycemia may be necessary. In this situation, any increase in the apparent glucose disposal rate may be accentuated by the difference between our experimental design and that described previously.[12] However, the difference between the forearm vein and arterial glucose concentrations has been reported to be only 15% under the steady-state condition of a hyperglycemic clamp.[12] Furthermore, in our study, orally administered DG-5128 enhanced in vivo glucose disposal by 66% above steady-state levels at the 2nd h, which was 2.5-fold higher than that of the placebo-treated group, and the difference between placebo and DG-5128 groups was obtained at the same steady-state plasma glucose and insulin concentrations. Therefore, even though the effect of DG-5128 on in vivo glucose disposal might be slightly overestimated, the conclusion is not invalidated.

DG-5128 might stimulate peripheral glucose utilization or suppress hepatic glucose output. To eliminate the latter possibility, it would have been preferable to have performed studies that included [^{3}H]glucose infusion. However, in our study, approval for the use of [^{3}H]glucose in human subjects was not received. It has been reported that hepatic glucose output in both nonobese and obese diabetic subjects was suppressed to <7% of the preinfusion level by infusing insulin at the rate of 42.6 mU · m^{-2} · min^{-1}.[6] In our study, by infusing insulin (0.77 mU · kg^{-1} · min^{-1}) together with somatostatin (80 ng · kg^{-1} · min^{-1}) and glucose (>3 mg · kg^{-1} · min^{-1}), steady-state plasma IRI levels at the 2nd and 4th h of infusion was kept at ~50 μU/ml in the control group as well as in the DG-5128–treated group, and plasma glucagon level was significantly suppressed, as described previously.[13] Therefore it is reasonable to expect that hepatic glucose output in both groups was significantly suppressed during the last 2 h. These results suggest that an increment in glucose disposal rate between the 2nd and 4th h (2.8 mg · kg^{-1} · min^{-1}) in DG-5128–treated subjects is not due to a further decrease in hepatic glucose output but mainly to an enhancement of in vivo peripheral glucose utilization.

In our study, oral administration of DG-5128 acutely enhanced in vivo glucose disposal. However, it was not clearly shown that the effect of DG-5128 on in vivo glucose disposal was mediated by α_2-adrenergic receptors. Interestingly, Cherksey and Altszuler[14] reported that glyburide displaced [^{3}H]clonidine (a specific α_2-adrenergic agonist) binding to islet adrenergic receptors, indicating that effects of glyburide on insulin release in subjects with NIDDM might be associated with the α_2-adrenergic receptor. Therefore the in vitro

effects of DG-5128, with or without catecholamine, on glucose utilization in peripheral tissues such as muscle cells, liver cells, and adipocytes must be elucidated. In conclusion, oral administration of DG-5128 effectively corrects postprandial hyperglycemia in subjects with NIDDM by enhancement of both insulin secretion and in vivo glucose utilization.

REFERENCES

[1] Kameda, K., Ono, S., and Abiko, Y.: Hypoglycemic action of 2-[2-(4,5-dihydro-1*H*-imidazol-2-yl)-1-phenylethyl]pyridine dihydrochloride sesquihydrate (DG-5128), a new hypoglycemic agent. Arzneim. Forsch. 1982; 32:39–44.

[2] Kawazu, S., Suzuki, M., Watanabe, T., Ishii, J., Nomura, H., and Hakusui, H.: Studies of DG-5128, a new type of hypoglycemic drug, in healthy subjects. Abstract. Diabetes 1984; 33 (Suppl.):87A.

[3] Kameda, K., Ono, S., Koyama, I., and Abiko, Y.: Insulin releasing action of 2-[2-(4,5-dihydro-1*H*-imidazol-2-yl)-1-phenylethyl]pyridine dihydrochloride sesquihydrate (DG-5128), a new orally effective hypoglycemic agent. Acta Endocrinol. 1982; 99:410–15.

[4] Kameda, K., Koyama, I., and Abiko, Y.: Comparative study on the effects of a hypoglycemic 2-substituted-2-imidazoline derivative (DG-5128) and tolbutamide on insulin secretion from and insulin synthesis in the isolated rat pancreatic islets. Biochim. Biophys. Acta 1981; 677:263–68.

[5] Harano, Y., Furusawa, S., Suzuki, M., Kosugi, K., Hidaka, H., Ohgaku, S., and Shigeta, Y.: Effect of treatment on insulin sensitivity in diabetic subjects. *In* Proceedings of the First International Symposium on Treatment of Diabetes Mellitus. Amsterdam, Excerpta Med., 1983:222–29.

[6] Reaven, G. M., Doberne, L., and Greenfield, M. S.: Comparison of insulin secretion and in vivo insulin action in non-obese and moderately obese individuals with non-insulin-dependent diabetes mellitus. Diabetes 1982; 31:382–84.

[7] Hoffman, W. S.: A rapid photoelectric method for the determination of glucose in blood and urine. J. Biol. Chem. 1937; 120:51–55.

[8] Hales, C. N., and Randle, P. J.: Immunoassay of insulin with antibody precipitate. Biochem. J. 1963; 88:137–46.

[9] Block, M. B., Mako, M. E., Steiner, D. F., and Rubenstein, A. H.: Circulating C-peptide immunoreactivity studies in normal and diabetic patients. Diabetes 1972; 21:1013–26.

[10] Trivelli, L. A., Ranney, H. M., and Lai, H. T.: Hemoglobin components in patients with diabetes mellitus. N. Engl. J. Med. 1971; 284:353–57.

[11] Doberne, L., Greenfield, M. S., Schulz, B., and Reaven, G. M.: Enhanced glucose utilization during prolonged glucose clamp studies. Diabetes 1981; 30:829–35.

[12] McGuire, E. A. H., Herderman, J. D., Andres, R., and Berman, M.: Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J. Appl. Physiol. 1976; 41:565–73.

[13] Harano, H., Hidaka, K., Takatsuki, O., Ohgaku, M., Haneda, S., Motoi, K., Kawagoe, Y., Shigeta, Y., and Abe, H.: Glucose, insulin, and somatostatin infusion for the determination of insulin sensitivity in vivo. Metabolism 1978; 27:1449–52.

[14] Cherksey, B., and Altszuler, N.: Tolbutamide and glyburide differ in effectiveness to displace α- and β-adrenergic radioligands in pancreatic islet cells and membranes. Diabetes 1984; 33:499–503.