Endocrine Journal 1996, **43** (1), 125–130

NOTE

Modified Method Using a Somatostatin Analogue, Octreotide Acetate (Sandostatin®) to Assess *in Vivo* Insulin Sensitivity

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Abstract. In order to evaluate the steady state plasma glucose (SSPG) method by using a new somatostatin derivative, octreotide acetate (Sandostatin®) instead of somatostatin that we had used for the insulin sensitivity test, we examined whether octreotide was able to suppress C-peptide (CPR), glucagon (IRG), and GH to a similar degree to that achieved with somatostatin. A total of 52 studies were performed in 45 essential hypertensive subjects and 7 healthy subjects. Octreotide was given subcutaneously in a does of 50 μg or 100 μg 10 min before the test (sc 50, sc 100 groups) or intravenously infused over 2 h (10 μg in bolus followed by a constant infusion, 50, 100, or 150 μg/2 h: iv 50, iv 100, iv 150 groups). In all of the groups the plasma immunoreactive insulin (IRI) concentration increased gradually after insulin injection and reached the steady state plasma insulin (SSPI) level between 40 and 60 μU/ml at 60 min through 120 min. Plasma CPR at 120 min was the most suppressed (by 67% of the basal level in iv 150 group during the study period), but on the other hand in both the sc 100 and iv 100 groups the plasma CPR concentration at 120 min was suppressed by nearly 40%, but not significantly suppressed in either the sc 50 or the iv 50 group. Plasma IRG and GH were strongly suppressed after 60 min in all the groups during the study period. Plasma glucose had increased significantly at 30 min and reached the steady state at 90 min through 120 min in hypertensive and healthy subjects. The results indicated that the modified SSPG method with continuous intravenous infusion of Octreotide at 150 μg/2 h was adequate for the measurement of insulin sensitivity.

Key words: Insulin sensitivity test, Steady State Plasma Glucose Method, Octreotide acetate, Sandostatin, Hypertension

(*Endocrine Journal* **43**: 125–130, 1996)

INSULIN resistance is known to be present in diabetes [1, 2], obesity [3] and hypertension [4–6]. Recently there has been increasing interest in insulin resistance as a possible risk factor for glucose intolerance, hypertension, hyperlipidemia and cardiovascular disease [7, 8]. Methods to evaluate *in vivo* insulin sensitivity are the glucose clamp, steady state plasma glucose (SSPG) method and that with the minimum model. We have previously reported the SSPG method with somatostatin in 1978 [9, 10]. This method is simple and suited for the repetitive evaluation of insulin sensitivity in the same subjects. In contrast to somatostatin, which is not commercially available, its analog, Octreotide, has been clinically used for the management of enterohormone producing tumor [11] and acromegaly [12]. In tis study, the dose & administration route of Octreotide have been evaluated to replace so-

Received: February 8, 1995
Accepted: October 19, 1995
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matostatin for the quantification of insulin sensitivity for glucose metabolism *in vivo*.

Materials and Methods

Patients

Seven healthy control & 45 non-obese hypertensive subjects, including those with borderline hypertension, were studied by this method. Hypertension was defined if either systolic or diastolic blood pressure measured when sitting position exceeded 140 and 90 mmHg, respectively. On 75 g oral glucose tolerance test (OGTT), 22 were normal, 11 IGT, 12 mildly diabetic with fasting plasma glucose (FPG) below 140 mg/d*l* in hypertensive subjects. They had not been treated with diuretics, nor β blocker which were known to deteriorate insulin sensitivity for hypertension and had no endocrine, renal, hepatic or other metabolic diseases.

Methods

Insulin sensitivity test: For the purpose of the study protocol to evaluate the suppressibility of endogenous insulin and its antagonistic hormones, the above heterogenous subjects were investigated for different doses of octreotide. The study started in the morning after an overnight fast for at least 10 h. The same method as previously reported [9, 10] was used except that somatostatin was replaced by long acting octreotide acetate (Sandostatin®, Sandoz. Ltd, Switzerland), which was subcutaneously injected (50, 100 μg) 10 min before the test, or intravenously infused over 2 h (10 μg in bolus followed by a constant infusion, 50, 100, 150 μg/2 h). Insulin (Novolin R 40®, Novo, Denmark) was infused with an insulin infusion pump (Nipro SP-10) at a rate of 0.77 mU/kg/min body weight with an initial bolus injection (15 mU/kg). Glucose (6 mg/kg/min) was infused through an antecubital vein in a 12% solution containing 5 meq KCL to prevent hypopotassemia. Blood samples were obtained basically every 30 min for 2 h for the determination of plasma glucose, immunoreactive insulin (IRI), C-peptide (CPR), GH, and glucagon (IRG). Glucose was determined by the enzymatic method and hormones by radioimmunoassay as described elsewhere.

Statistical analysis

Values are given as the means ± SEM. The significance of the mean difference between the patient profiles in each group was determined by Scheffe's *t*-test. Paired *t*-test was used for the glucose, IRI, CPR, GH and IRG concentrations before and after octreotide infusion.

The study method was approved by the hospital ethics committee and informed consent was obtained from each subject.

Results

Patients' profiles for the SSPG method are shown in Table 1. There was no difference among five groups (50 μg and 100 μg subcutaneously; 50 μg, 100 μg, and 150 μg intravenously) as to sex, age, body mass index (BMI), systolic or diastolic blood pressure (SBP, DBP), FPG, basal IRI and the area under the curve of plasma glucose and IRI levels during the 75 g oral glucose tolerance test. Plasma IRI increased gradually after insulin injection and reached the steady state at from 60 min to 120 min in all groups (Fig. 1). Steady state plasma insulin (SSPI) levels were between 40 and 60 μU/m*l* in all of the administration routes of octreotide and the SSPI levels of iv 150 group were smaller than the other groups but not significantly. As shown in Fig. 2, plasma CPR levels significantly reduced at 30 min in all groups, but in cases of the sc 50 and iv 50 groups, plasma CPR increased gradually after 30 min and there was no difference between the values at 0 and 120 min, suggesting inadequate suppression of endogenous insulin secretion. In the other two groups (sc 100 and iv 100) plasma CPR slightly increased after 60 min and the levels at 120 min were partially suppressed by 38% and 37% of each basal level. Plasma CPR in the intravenous administration of 150 μg octreotide was the most suppressed by 67% of the basal level at 120 min. As shown in Figs. 3 and 4, plasma IRG and GH were significantly suppressed after 30 min in all groups during the insulin sensitivity test. As shown in Fig. 5, plasma glucose significantly had increased at 30 min and reached the steady state at from 90 min to 120 min in all groups. The SSPG level of five normotensive healthy control subjects in the two (sc 100 and iv 100) groups was 96 ± 15 mg/d*l*.

Table 1. Patient profile of insulin sensitivity test

		sc 50 group	sc 100 group	iv 50 group	iv 100 group	iv 150 group
Total number		4	29	4	10	5
Hypertension		3	26	3	8	5
(DM/IGT/NGT)		(0/1/2)	(7/8/11)	(0/1/2)	(3/1/4)	(2/0/3)
Normal healthy control		1	3	1	2	0
Sex	(M/F)	(2/2)	(20/9)	(4/0)	(3/7)	(2/3)
Age	(yrs)	50 ± 8	58 ± 3	51 ± 4	53 ± 4	50 ± 5
BMI	(kg/m^2)	23 ± 2	23 ± 1	23 ± 1	22 ± 1	23 ± 2
SBP	(mmHg)	142 ± 13	143 ± 3	157 ± 13	140 ± 6	148 ± 14
DBP	(mmHg)	87 ± 13	82 ± 3	100 ± 7	86 ± 4	93 ± 7
FPG	(mg/dl)	109 ± 18	104 ± 3	98 ± 6	111 ± 8	103 ± 7
IRI	(μU/ml)	7 ± 1	7 ± 1	5 ± 2	6 ± 1	8 ± 3
$\sum_{0}^{120}G$	(mg/dl h)	349 ± 43	348 ± 17	305 ± 45	354 ± 34	364 ± 61
$\sum_{0}^{120}I$	(μU/ml h)	86 ± 17	85 ± 2	88 ± 14	93 ± 6	72 ± 13

Mean ± SEM. sc 50, sc 100, Sandostatin was given subcutaneously in dose of 50 μg or 100 μg. iv 50, iv 100, iv 150, Sandostatin was given intravenously in dose of 50 μg, 100 μg, or 150 μg over 2 h. DM, diabetes mellitus; IGT, Impaired glucose tolerance; NGT, normal glucose tolerance; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; IRI, immunoreactive insulin.

$\sum_{0}^{120}G$, $\sum_{0}^{120}I$: Area under the curve of glucose and insulin levels during the 75 g oral glucose tolerance test.

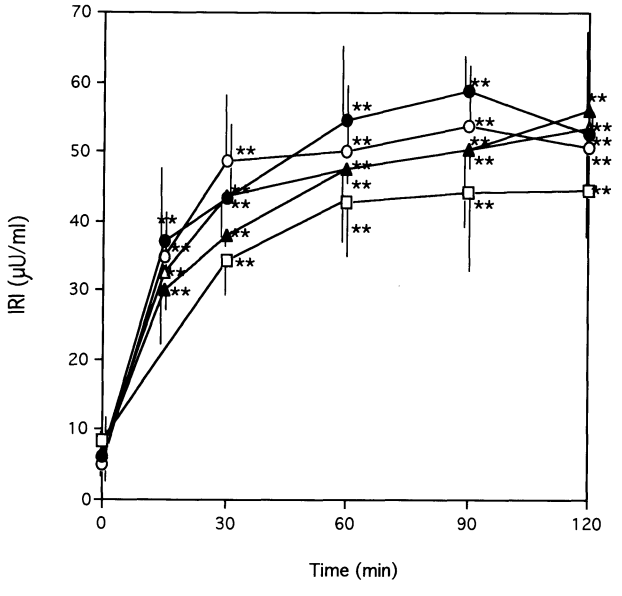

Fig. 1. Plasma IRI concentrations during the insulin sensitivity test with various doses of octreotide. ** $P<0.01$ as compared with time 0 by paired t-test. -△- sc 50 μg (n=4), -▲- sc 100 μg (n=29), -○- iv 50 μg (n=4), -●- iv 100 μg (n=10), -□- iv 150 μg (n=5). sc, subcutaneous infusion of octreotide; iv, intravenous infusion of octreotide.

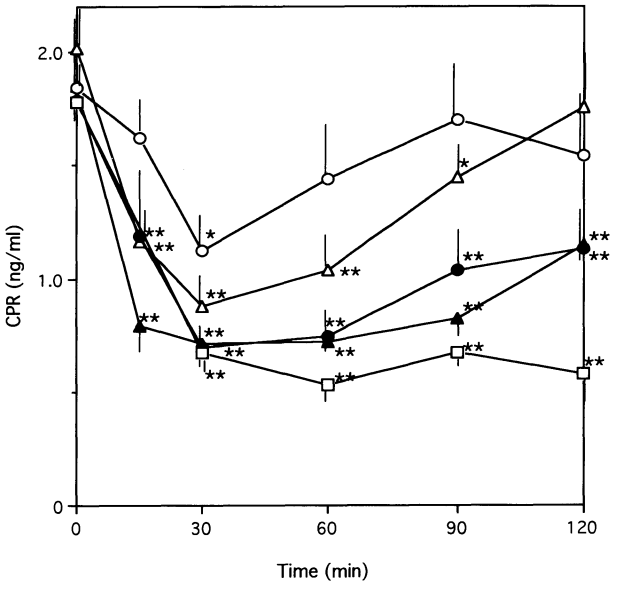

Fig. 2. Plama CPR concentrations during the insulin sensitivity test with various doses of octreotide. * $P<0.05$, ** $P<0.01$ as compared with time 0 by paired t-test. -△- sc 50 μg (n=4), -▲- sc 100 μg (n=29), -○- iv 50 μg (n=4), -●- iv 100 μg (n=10), -□- iv 150 μg (n=5). sc, subcutaneous infusion of octreotide; iv, intravenous infusion of octreotide.

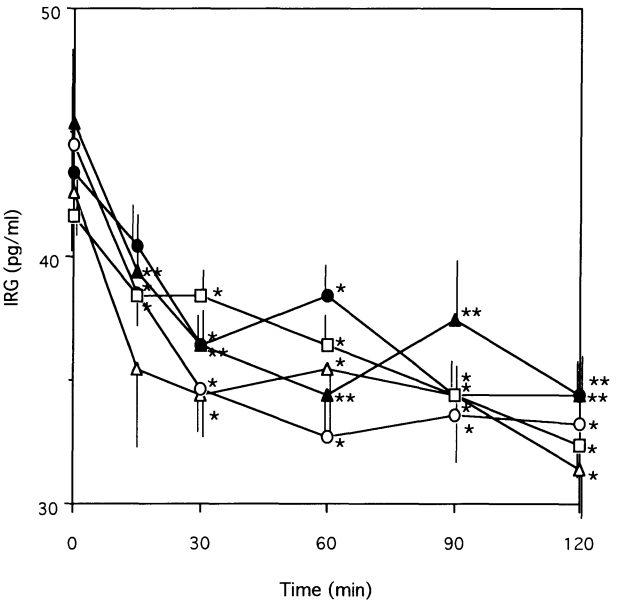

Fig. 3. Plasma IRG concentrations during the insulin sensitivity test with various doses of octreotide. * $P<0.05$, ** $P<0.01$ as compared with time 0 by paired t-test. -△- sc 50 μg (n=4), -▲- sc 100 μg (n=29), -○- iv 50 μg (n=4), -●- iv 100 μg (n=10), -□- iv 150 μg (n=5). sc, subcutaneous infusion of octreotide; iv, intravenous infusion of octreotide.

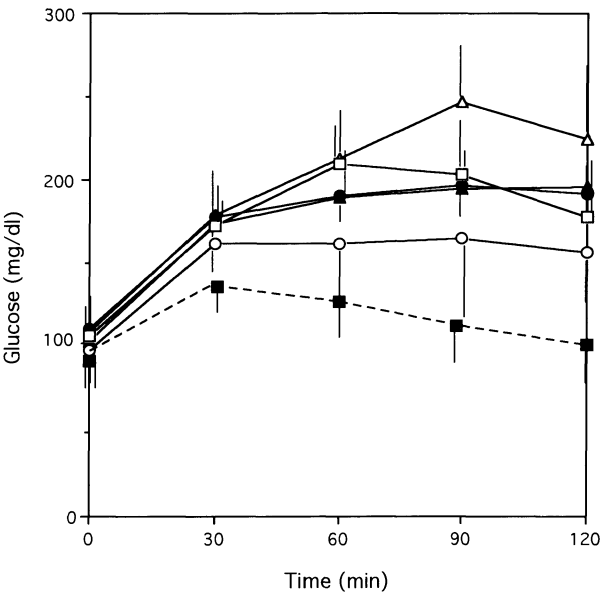

Fig. 5. Plasma glucose concentrations during the insulin sensitivity test with various doses of octreotide. -△- sc 50 μg (n=4), -▲- sc 100 μg (n=29), -○- iv 50 μg (n=4), -●- iv 100 μg (n=10), -□- iv 150 μg (n=5), --■-- normotensive healthy control in both sc 100 and iv 100 groups (n=5). sc, subcutaneous infusion of octreotide, iv, intravenous infusion of octreotide.

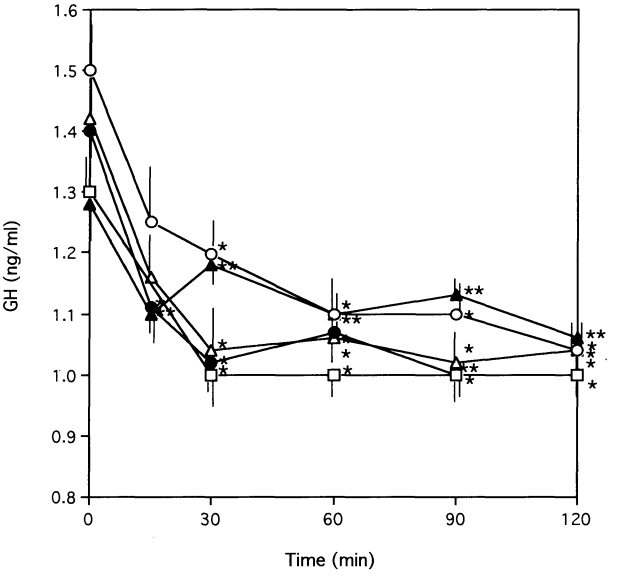

Fig. 4. Plasma GH concentrations during the insulin sensitivity test with various doses of octreotide. * $P<0.05$, ** $P<0.01$ as compared with time 0 by paired t-test. -△- sc 50 μg (n=4), -▲- sc 100 μg (n=29), -○- iv 50 μg (n=4), -●- iv 100 μg (n=10), -□- iv 150 μg (n=5). sc, subcutaneous infusion of octreotide; iv, intravenous infusion of octreotide.

Discussion

We had previously modified the SSPG method originally reported by Shen *et al.* using somatostatin [13]. The recently developed cyclic octapeptide Sandostatin®, with the amino-acid sequence (D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr(ol), is an analogue of somatostatin that has a longer duration of action than somatostatin and seems to be more potent than the natural compound in its inhibition of gut-hormone secretion [14]. We now report the newly developed method replacing somatostatin by octreotide, since the latter is commercially available.

In essential hypertension plasma IRG and GH levels were well suppressed by intravenous and subcutaneous infusion of octreotide in every dose above 50 μg. Among the five groups there was no difference in the endogenous insulin secretion when evaluated by the area under the curve of the plasma IRI concentration on 75 g OGTT. Plasma CPR could be partially suppressed by almost 40% of the basal level by intravenous or subcutaneous

infusion of more than 100 μg octreotide and was kept below 0.6 ng/ml by intravenous infusion of 150 μg octreotide. Plasma IRI reached its peak between 40 and 60 μU/ml at from 60 min to 120 min and the concentrations were about the same as the physiological meal-stimulated concentration often observed in hypertensive or obese subjects in our country. The SSPI concentrations in the iv 150 group were smaller than the other groups probably because endogenous insulin secretion would be most suppressed in this condition. The above results therefore suggested that infusion of octreotide intravenously at a 150 μg dose was adequate for the determination of SSPG levels. The relative potency for the suppression of IRI compared with IRG & GH seemed weaker with octreotide than with somatostatin. The proper dose of octreotide should therefore be used for the practically sufficient suppression of endogenous insulin secretion (CPR) in the SSPG method. Plasma glucose increased at 30 min and reached the steady state at from 90 to 120 min. These data indicated that measuring of plasma glucose level at 120 was adequate for the evaluation of steady state plasma glucose levels.

Regarding side effects, at low doses (25 μg and 50 μg intravenously; 50 μg and 100 μg subcutaneously) no side effect was noted. Short-lasting nausea or stomachache was reported after the injection of octreotide (100 μg) injected intravenously [14]. We have not observed any adverse effects of the injection of octreotide (50 and 100 μg subcutaneously; 50, 100, and 150 μg intravenously) during the insulin sensitivity test. But after the completion of this study, we noted diarrhea and acholic stool in 2 cases out of 188 at a 150 μg dose during a 2 h test, probably due to the suppression of bile acid and digestive enzyme secretion and intestinal movement [14]. The prescription of anti-laxatives and digestive enzymes when necessary may be indicated after the test.

A number of papers have shown that in essential hypertension insulin resistance has been present [4, 5]. The SSPG concentrations of essential hypertension without glucose intolerance in the three combined groups {161 ± 14 mg/dl, n=18: sc 100 (n=11), iv 100 (n=4), and iv 150 (n=3)}, except the last two groups (sc 50, iv 50) for incomplete IRI suppression, were significantly higher ($P<0.05$) than in normotensive healthy subjects {96 ± 15 mg/dl, n=5; sc 100 (n=3), and iv 100 (n=2)}. There was no difference between the two groups in age, sex, BMI, FPG or IRI. The SSPG concentrations in these normotensive healthy subjects were almost the same as those we had reported with somatostatin [6]. We have reported with the SSPG method that hypertension has been more closely associated with insulin insensitivity than hyperinsulinemia [15] and that this insensitivity has been partially reversible by α_1-blocker bunazosin [6], and long-acting Ca antagonist amlodipine [16]. These results indicated that inadequate insulin action, rather than hyperinsulinemia, might play an important role in the pathophysiology of hypertension. The clinical implication of insulin resistance is that diabetic subjects tend to develop hypertension more easily.

In conclusion, the SSPG method with continuous intravenous infusion of octreotide acetate at 150 μg/2 h is adequate for the suppression of endogenous insulin and its antagonistic hormone and will be helpful for the quantification of insulin resistance.

Acknowledgment

This study was supported in part by Special Coordination Funds for Promoting Science and Technology (Encouragement System of COE) from the Science and Technology Agency of Japan and a grant for Scientific Research Expenses for Health and Welfare Programs (Clinical Treatment of Diabetes Mellitus, Akanuma).

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