The effect of low dose octreotide administration on renal function and on gene expression of IGF-I axis components in experimental diabetes mellitus

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Abstract

The present study was undertaken to assess the chronic effects of low dose octreotide (Oc) administration in rats with experimental diabetes mellitus (DM). Metabolic and clearance studies were performed in control normal rats, in rats with streptozotocin-induced DM of 1 week duration and in similar DM rats treated with Oc, 10–20 µg/day. Gene expression of IGF-I, IGF-I receptor (IGF-I R) and IGF-binding protein-1 (IGFBP-1) was examined in renal tissue from normal DM animals and DM animals treated with Oc 10, 20 and 100 µg/day.

Seven days of Oc administration, 10 µg/day, in rats with experimental DM, was associated with enhanced hyperglycemia, increased glomerular filtration rate and urinary sodium excretion as compared with untreated DM animals. After a higher Oc dose, 20 µg/day, however, there were no significant changes in renal function and in glycemic control. Significant increases in kidney weight and kidney weight/body weight ratio were seen in DM rats as compared with control intact animals. These

changes were not affected by Oc therapy in various doses.

Induction of DM was associated with a marked increase in renal IGFBP-1 mRNA expression. There were no significant changes in the expression of IGF-I or IGF-I R mRNA. Oc therapy in a low or high dose did not affect gene expression of IGF-I, IGF-I R or IGFBP-1.

Thus, the response to chronic low dose Oc administration of DM rats may vary from enhanced hyperglycemia and hyperfiltration to a lack of change in renal function or in glycemic control. Low dose Oc therapy was not associated with significant variations in renal mass or in the gene expression of IGF-I axis components.

These findings are at variance with previously published studies which show a suppressive effect of Oc on renal function and growth in experimental diabetes. This apparent discrepancy may be related to the duration of treatment or to a biphasic physiological effect of Oc when used in different doses.

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Introduction

Diabetic nephropathy is characterized by an early increase in kidney size and function, with subsequent mesangial proliferation, extracellular matrix accumulation, albuminuria and glomerular sclerosis (Parving *et al.* 1996). In the last decade a significant amount of research in humans and laboratory animals has been dedicated to the role of the insulin-like growth factor-I/growth hormone (IGF-I/GH) system in the pathogenesis of diabetic renal disease. The most frequently studied animal model was streptozotocin (STZ)-induced diabetes in rats. In this model, STZ administration is associated with insulopenia, hyperglycemia, kidney enlargement and hemodynamic changes similar to those seen in insulin dependent diabetes in humans (Seyer-Hansen 1976, Jensen *et al.* 1981, Wald & Popovtzer 1984). These effects could be mimicked by GH and/or IGF infusion in both human and rats (Jensen *et al.* 1981, Guler *et al.* 1988, 1989, Flyvbjerg *et al.* 1991).

Furthermore, infusion of IGF-I in rats with experimental diabetes mellitus (DM) was associated with enhanced renal hypertrophy (Flyvbjerg *et al.* 1991), suggesting that IGF-I is a major factor in these processes. A role for growth factors in the induction of renal hypertrophy and glomerulosclerosis was also shown in studies using transgenic mice (Doi *et al.* 1988). These animals, expressing exaggerated levels of either IGF-I or GH, display renal and glomerular hypertrophy. Transgenic mice expressing elevated GH but not IGF-I levels develop morphological lesions similar to those of diabetic glomerulosclerosis despite the absence of hyperglycemia or hypertension (Doi *et al.* 1988).

Induction of DM with STZ in rats is associated with a marked rise in renal IGF-I. This increase reaches a peak value 1–28 h after the onset of DM and precedes the increase in the kidney weight (Flyvbjerg *et al.* 1988, 1989). The increase of renal IGF-I tissue content in early DM is probably not the result of enhanced synthesis, since

the expression of IGF-I mRNA usually declines 2 to 7 days after the onset of the diabetic state (Fagin *et al.* 1989, Flyvbjerg *et al.* 1990). It was therefore suggested that sequestration of IGF-I due to elevated levels of IGF-I-binding proteins, rather than increased production, accounts for the marked kidney IGF-I accumulation in early DM (Phillip *et al.* 1994).

The long acting somatostatin analog, octreotide (Oc) is a potent suppressor of GH secretion and has been used in DM to modulate the glycemic control via the modulation of the GH/IGF-I system and reduction of insulin resistance (Ørskov *et al.* 1992, Schulkes 1994). It was also shown that Oc administration is associated with a decrease in renal IGF-I content and a significant suppression of kidney growth at both short-term and long-term follow-up (Flyvbjerg *et al.* 1988, 1989, 1992). In these studies, however, Oc was administered in a pharmacological dose, ten times greater than that normally used in the clinical setting.

In contrast to the above findings, less consistent results were reported when Oc was administered in lower doses. In one of these studies, low dose Oc therapy (in rats with aloxan-induced diabetes) was associated with reduced kidney weight and suppressed activity of the pentose phosphate pathway as compared with untreated diabetic animals. In this investigation data on renal function were not presented (Steer *et al.* 1988).

In another experiment, long-term Oc administration in a low dose was studied in diabetic rats after unilateral nephrectomy. This model is usually characterized by accelerated glomerulosclerosis as compared with that seen in animals after classical STZ-induced DM. In this study Oc therapy did not affect the hemodynamic or morphological alteration associated with combined diabetes and uninephrectomy (Muntzel *et al.* 1992).

The present study was undertaken to reassess the chronic effects of low dose Oc administration in rats with STZ-induced DM. Metabolic and clearance studies were performed in rats with experimental DM and in normal animals. IGF-I, IGF-I receptor (IGF-I R) and insulin-like growth factor-binding protein-1 (IGFBP-1) mRNAs were examined in renal tissue from control rats, rats with STZ-induced DM and rats with DM treated with Oc.

Materials and Methods

Experimental protocol

Male rats of the Hebrew University Sabra strain weighing 200–220 g were used in all experiments. The animals were placed in individual metabolic cages with free access to tap water and standard rat chow.

Two series of investigations were performed.

The first series was the effect of Oc on renal function in rats with STZ-induced DM. These metabolic studies were performed in two subsets using two different doses of Oc.

In the First Study three groups of rats were used: (1) normal rats ($n=7$), (2) rats with STZ-induced DM ($n=14$), and (3) rats with STZ-induced DM treated with Oc (Sandostatin, Somatostatin analog, Sandoz) 5 µg subcutaneously twice a day ($n=13$). In the Second Study, two groups of rats were used: (1) rats with STZ-induced DM ($n=5$), and (2) rats with STZ-induced DM treated with Oc 10 µg subcutaneously twice a day ($n=7$). The effects of Oc administration in normal rats was also studied. In this experiment, the following groups were studied: (1) normal rats ($n=12$), and (2) normal rats treated with Oc 5 µg twice a day subcutaneously.

The second series was a determination of IGF-I, IGF-I R and IGFBP-1 mRNA. In this series, called the Third Study, determinations of IGF-I, IGF-I R and IGFBP-1 mRNA were performed in separate experiments in control animals, rats with STZ-induced DM and rats with STZ-induced DM treated with Oc, 10 and 20 µg/day (given in two subcutaneous doses as described). After initial experiments failed to show a significant effect of Oc (see results), an additional study was performed using an Oc dose of 100 µg/day. Following completion of the experimental protocol, the rats were killed by aortic puncture exsanguination under ether anesthesia. The kidneys were decapsulated, and equal quadrants were immediately frozen in liquid nitrogen for RNA extraction.

Induction of DM and Oc treatment Diabetes mellitus was induced by subcutaneous injection of STZ, 55 mg/kg body weight (BW) dissolved in citrate buffer 0.01 M (pH 4.5). Control (non-diabetic) animals were injected with citrate buffer in a volume similar to that used in diabetic rats. Treatment with Oc lasted 1 week in both subsets. Diabetic and non-diabetic rats that were not treated with Oc were injected with similar volumes of saline. Fluid and food intake, urine output, plasma and urinary creatinine, sodium, potassium and urinary albumin excretion were checked in all animals from the First and the Second Study. Plasma glucose and insulin levels were checked in all animals (First, Second and Third Studies).

At the end of all experiments kidney weight and kidney weight/BW ratio were compared in all subgroups.

Insulin determination

Plasma insulin levels were determined by RIA using human insulin as standard (Sorin Biomedica, Salluggia, Italy).

Tissue mRNA assay

The assays for IGF-I, IGF-I R, and IGFBP-1 were performed by a solution hybridization-RNase protection assay as follows. Total RNA was prepared from the renal tissue of individual rats by Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) according

Table 1 The effect of Oc administration (10 µg/day, First Study, and 20 µg/day, Second Study) in rats with STZ-induced (DM). Data shown are means ± S.E.M.

	First Study			Second Study	
	Control (n=7)	DM (n=14)	DM+Oc 10 (n=13)	DM (n=5)	DM+Oc 20 (n=7)
Body weight (g)	226 ± 14	220 ± 8	210 ± 6	209 ± 10	210 ± 6
U _v (ml/24 h)	11 ± 1	48 ± 9 ^a	80 ± 8 ^{a,d}	49 ± 19 ^a	41 ± 16 ^a
Fluid intake (ml/24 h)	35 ± 3	72 ± 11 ^a	107 ± 9 ^{a,d}	72 ± 24 ^a	65 ± 18 ^a
Food intake (g/24 h)	23 ± 3	20 ± 3	27 ± 3	26 ± 4	24 ± 3
P glucose (mmol/l)	9.1 ± 0.2	25.0 ± 2.7 ^a	31.0 ± 1.6 ^{a,e}	24.8 ± 5.7 ^a	22.4 ± 4.6 ^a
P insulin (mIU/ml)	98.4 ± 9.7	32.8 ± 5.1 ^a	57.3 ± 3.2 ^{c,e}	24.9 ± 4.9 ^a	21.7 ± 2.2 ^a
P _{cr} (µmol/l)	62.8 ± 2.3	63.7 ± 2.4	63.7 ± 1.5	70.2 ± 5.6	66.7 ± 4
C _{cr} (µl/min/100 g BW)	364 ± 17	422 ± 31 ^c	517 ± 24 ^{c,e}	445 ± 30 ^c	432 ± 31 ^c
U _{Na} V (mEq/24 h)	1.22 ± 0.01	1.58 ± 0.13 ^b	1.90 ± 0.09 ^{b,e}	1.87 ± 0.33 ^c	2.58 ± 1.06 ^a
U _K V (mEq/24 h)	2.13 ± 0.15	3.68 ± 0.27 ^b	4.49 ± 2.4 ^b	3.46 ± 0.77	2.93 ± 0.47 ^c
U albumin (mg/24 h)	5.7 ± 0.6	15.2 ± 2.4 ^b	20.4 ± 2.4 ^b	10.2 ± 2.4 ^a	9.9 ± 3.2 ^c
Kidney weight (g)	1.63 ± 0.09	2.07 ± 0.08 ^b	2.17 ± 0.06 ^b	1.95 ± 0.15 ^c	1.83 ± 0.04 ^b
Kidney weight/BW × 10 ³	7.66 ± 0.61	9.41 ± 0.31 ^c	10.33 ± 0.30 ^c	9.29 ± 0.15 ^c	8.81 ± 0.21

^aP<0.0025 vs control, ^bP<0.005 vs control, ^cP<0.05 vs control, ^dP<0.01 vs DM, ^eP<0.05 vs DM.

U_v, urine volume; P, plasma; C_{cr}, creatinine; U_{Na}V, urinary sodium excretion; U_KV, urinary potassium excretion; U albumin, urinary albumin excretion.

to the method of Chomczynski (1993) and quantified by absorbance at 260 nm. The integrity of the RNA and the accuracy of the spectrophotometric determinations were assessed by visual inspection of the ethidium bromide-stained 28S and 18S ribosomal RNA bands after agarose formaldehyde gel electrophoresis of 10 µg aliquots as described previously (Lowe *et al.* 1987b). The IGF-I, IGF-I R, and IGFBP-1 riboprobes were generous gifts from Derek LeRoith (NIH, Bethesda, MD, USA).

The antisense RNA probe used to detect IGF-I R mRNA has been previously described (Werner *et al.* 1989). This transcript contains 40 bases of vector sequence and 265 bases complementary to 15 bases of 5'-untranslated sequence and to the region encoding the signal peptide and the first 53 amino acids of the IGF-I R α-subunit. On hybridization of this RNA probe with IGF-I R RNA and subsequent RNAase digestion, a protected band of 265 bases was obtained.

The riboprobe used to measure the levels of IGF-I mRNA was described previously (Lowe *et al.* 1988). This probe allows the detection of both IGF-I mRNA species encoding the IGF-Ia and IGF-Ib prohormones. Only the level of IGF-Ia mRNA, which constitutes >90% of the total IGF-I message and correlates with the levels of IGF-Ib mRNA, was measured in this study. The IGFBP-1 mRNA was measured with an antisense probe derived from a rat IGFBP-1 cDNA clone isolated from a dexamethasone-treated H-4-11-E-C3 hepatoma cell library (G T Ooi, unpublished observations). The size of the protected band obtained by hybridizing this antisense RNA probe with IGFBP-1 mRNA was 203 bases.

The PT7 RNA 18S (Ambion, Austin, TX, USA) was used as an antisense control. The size of the protected fragment was 80 bases.

Solution hybridization-RNAase protection assays were performed as described (Lowe *et al.* 1987a). Briefly, 20 µg of total RNA were hybridized with 1 × 10⁶ d.p.m. ³²P-labeled antisense RNA probes. One nanogram of total RNA was hybridized with 18S antisense RNA probe. The hybridization was carried out at 45 °C for 16 h in a buffer containing 80% formamide. After hybridization, RNA samples were digested with RNAase A and T1 and the protected hybrids were extracted with phenol-chloroform, ethanol precipitated, and electrophoresed on 8% polyacrylamide-8 M urea denaturing gel. Multiple autoradiograms from each gel were scanned by a densitometer connected to a MacIntosh computer (Apple Computer Inc., Cupertino, CA, USA).

Statistical evaluation

Statistical evaluation was performed using ANOVA, an unpaired *t*-test and an unpaired *t*-test with Bonferroni correction for multiple comparison when indicated.

Results

The effect of Oc administration on renal function in rats with STZ-induced DM

Table 1 shows the renal and the metabolic effects of Oc administration 10 µg/day (First Study) or 20 µg/day (Second Study) in rats with STZ-induced DM. Several effects of Oc administration, 10 µg/day, are also depicted in Fig. 1. Induction of diabetes was associated with polydipsia, polyuria and significant increase in total kidney (two kidneys) weight as compared with the control

Figure 1 Creatinine clearance (µl/min per 100 g BW), urinary sodium and glucose excretion and plasma glucose in control rats, in rats with DM and with DM treated with Oc, 10 µg/day (DM+Oc). * and ** denote statistically significant differences vs control and DM respectively. For details, see Table 1.

animals. Polydypsia and polyuria, but not changes in kidney mass, were more prominent in rats treated with Oc. There were no significant changes in food intake between groups.

The creatinine clearance and the urinary sodium excretion were significantly increased in the diabetic rats (Table 1). These changes were more prominent in the diabetic animals treated with Oc, 10 µg/day. In these animals plasma glucose and the urinary glucose excretion were higher than in the untreated DM rats. Plasma insulin levels were 98.4 ± 9.7 mIU/ml in controls. In the First Study they were 32.8 ± 5.1 mIU/ml in DM rats and 57.5 ± 3.2 mIU/ml in animals treated with Oc, 10 µg/day (*P* < 0.0025 and 0.05 vs control). In the Second Study they were 24.8 ± 5.7 mIU/ml in DM and 22.4 ± 4.6 mIU/ml in animals treated with Oc, 20 µg/day (*P* < 0.0025 vs control).

No significant differences in plasma creatinine, sodium and potassium were seen between groups. DM was associated with enhanced potassium excretion, more prominently in rats treated with Oc, and with albu-

Table 2 The effect of low dose Oc administration (5 µg × 2/day) in normal rats. Data shown are means ± S.E.M.

	Control (n = 12)	Oc (n = 13)
Body weight (g)	229 ± 6.9	228 ± 2.1
Food intake (g/24 h)	21 ± 1.05	17.5 ± 0.77 ^a
U _v (ml/24 h)	14.00 ± 1.40	12.15 ± 1.91
Total kidney weight (g)	2.11 ± 0.07	2.11 ± 0.04
P glucose (mmol/l)	7.66 ± 0.24	8.31 ± 0.29
U glucose (mmol/24 h)	8.97 ± 0.91	8.36 ± 0.78
P _{cr} (µmol/l)	54.76 ± 1.39	50.88 ± 0.82 ^a
C _{cr} (µl/min/100 g BW)	454 ± 11	453 ± 19
U _{Na} V (mEq/24 h)	1.132 ± 0.120	1.201 ± 0.064
U _K V (mEq/24 h)	2.612 ± 0.085	2.320 ± 0.125

^a*P* < 0.05 vs control.
For abbreviations, see Table 1.

minuria. There was no significant difference in urinary albumin excretion between untreated diabetic rats and those treated with Oc (Table 1).

The renal function tests were similar in untreated rats with STZ-induced DM and DM rats treated with a higher dose of Oc, 20 µg/day (Second Study). There were no differences between these two groups with regard to BW, plasma glucose, food and fluid intake (Table 1).

It is worthwhile mentioning that kidney weight and kidney weight/BW ratio, significantly elevated in diabetic animals, were not affected by Oc therapy in high or low doses (Table 1).

Table 2 shows the effect of low dose (10 µg/day) Oc administration in normal animals. Oc therapy was associated with a mild decrease in food intake without significant changes in BW, renal function or kidney weight.

The effect of Oc administration on IGF-I, IGF-I R and IGFBP-1 gene expression in renal tissue (the Third Study)

Oc administration had no effect on renal IGF-I, IGF-I R or IGFBP-1 mRNA in normal rats (data not shown).

Figures 2 and 3 depict IGF-I, IGF-I R and IGFBP-1 in control animals, rats with experimental DM and diabetic animals treated with Oc 10 µg/day. Figures 4 and 5 depict IGF-I, IGF-I R and IGFBP-1 in control animals, rats with experimental DM and diabetic animals treated with Oc, 20 and 100 µg/day. The highest dose of Oc was administered after initial studies using 10 and 20 µg/day doses failed to disclose significant effects. Induction of diabetes (1 week duration) was not associated with significant changes in IGF-I or IGF-I R gene expression (Figs 2 and 4). IGFBP-1 was significantly increased in DM animals, but was not affected by Oc administration (Figs 3 and 5). IGF-I or IGF-I R gene expression were not effected by Oc in high or low doses.

Figure 2 The effect of Oc on IGF-I and IGF-I R gene expression in control rats (C), in DM rats and DM rats treated with Oc (10 µg/day). IGF-I and IGF-I R mRNA were measured by the solution hybridization-RNAase protection assay as described in Materials and Methods. Densitometric analysis did not show significant differences between groups.

Figure 3 The effect of Oc on IGFBP-1 gene expression in control rats (C), DM rats and in DM rats treated with Oc (10 µg/day). Renal levels of IGFBP-1 mRNA were measured by the solution hybridization RNAase protection assay as described in Materials and Methods. Densitometric analysis showed that IGFBP-1 mRNA expression increased, in DM to $179 \pm 32\%$ and in DM+Oc 10 to $200 \pm 32\%$ of control values ($P < 0.05$ vs control). There were no statistically significant differences between untreated and treated DM animals.

Figure 4 The effect of Oc on IGF-I and IGF-I R gene expression in control rats (C), in DM rats and in DM rats treated with Oc (20 or 100 µg/day). Renal levels of IGF-I and IGF-I R mRNA were measured by the solution hybridization RNAase protection assay as described in Materials and Methods. Densitometric analysis showed no significant differences between groups.

There were no significant differences in plasma glucose, insulin levels, kidney weight or kidney weight/BW ratio in DM rats treated with Oc 20 or 100 µg/day as compared with untreated DM animals.

Figure 5 The effect of Oc on IGFBP-1 gene expression in control rats, in DM rats and in DM rats treated with Oc (20 or 100 µg/day). Renal levels of IGFBP-1 mRNA were measured by the solution hybridization RNAase protection assay as described in Materials and Methods. Densitometric analysis showed that IGFBP-1 mRNA expression in DM animals increased to $149 \pm 13\%$, in DM+Oc 20 to $182 \pm 32\%$ and in DM+Oc 100 to $170 \pm 6\%$ of controls ($P < 0.05$ vs control). There were no statistically significant differences between untreated and Oc-treated animals.

Discussion

Our study shows that in rats with STZ-induced DM, Oc administration in a dose similar to that used in a clinical setting (10 µg/day) is associated with enhanced hyperglycemia, increased glomerular filtration rate (GFR) and enhanced natriuresis as compared with untreated DM animals. Paradoxically the insulin levels, markedly diminished by the diabetic state, were higher in rats treated with Oc, 10 µg/day, than in the untreated rats.

After a higher dose of Oc administration (20 µg/day) there were no significant changes in the renal function, kidney weight or the glycemic control. No significant effects were noted in normal animals treated with Oc.

Examination of the IGF-I axis components showed an increased expression of IGFBP-1 mRNA in renal tissue of DM animals. There were no significant changes in IGF-I or IGF-I R mRNA expression. These findings were not affected by Oc therapy, in low or high doses.

Since the early reports of the high IGF-I content found in the renal tissue of rats with experimental DM, many studies, short and long term, have been designed to assess the effect of somatostatin or its analog Oc, a potent suppressor of the GH/IGF-I system, on kidney function and mass in the diabetic state.

While it is generally accepted that Oc may diminish the insulin requirements and may improve the metabolic control in DM, its renal effects in DM with regard to increased GFR and renal size are controversial. These effects seem to be dependent on the magnitude of Oc dose, the duration of DM and the timing of the initiation of therapy relative to the duration of diabetes.

Flyvbjerg and others (Groenbaek *et al.* 1995a,b, Flyvbjerg *et al.* 1992), showed that Oc administration (200 µg/day) in rats with STZ-induced DM is associated with a significant suppression of kidney growth at both short- and long-term follow-up, especially following early intervention, i.e. 3 days or less of a diabetic state. Oc

administration in established diabetic nephropathy was unable to suppress the increase in the renal and glomerular volume or urinary albumin excretion (Groenbaek *et al.* 1995a,b).

In contrast, in a model of accelerated glomerulosclerosis combining experimental diabetes with unilateral nephropathy, chronic Oc therapy did not affect glomerular hyperfiltration, urinary protein excretion or renal histological changes associated with diabetes. In that study Oc was administered in a dose similar to that used for long-term therapy in clinical settings (30 µg/rat) (Muntzel *et al.* 1992).

Controversial data on the renal effects of Oc emerge also from clinical studies performed in diabetic patients. In patients with insulin-dependent diabetes mellitus treated with Oc (300 µg/day), decreases in GFR and kidney volume, assessed by high resolution computed tomography, were found after 12 weeks of therapy (Serri *et al.* 1991). By contrast, in another study, no statistically significant changes were noted in GFR after somatostatin analog administration (Krempf *et al.* 1990).

A possible mechanism of action of Oc in diabetic nephropathy implies a direct action of the analog on IGF-I axis components. Thus it was speculated that the effect of somatostatin and its analogs is related to reduction of serum concentration of free IGF-I through stimulation of IGFBP-1 release into the circulation following Oc administration, as shown in normal and acromegalic patients (Ezzat *et al.* 1991, Serri *et al.* 1991, Fredstorp *et al.* 1994, Ørskov *et al.* 1994).

In a preliminary study from our laboratory (Rubinger *et al.* 1994), however, Oc administration in a pharmacological dose (200 µg/day) in early (48 h) DM was associated with suppressed IGFBP-1 mRNA in both the cortex and the medulla as compared with untreated diabetic animals, but had no effect, however, on the renal function or on the renal mass.

In the present study performed on whole kidney tissue, the diabetic state (1 week duration) was not associated with significant changes in whole kidney IGF-I or IGF-I R mRNA while renal IGFBP-1 mRNA expression was enhanced. The finding regarding the unchanged IGF-I mRNA expression in DM rats relative to control animals is apparently at variance with other published studies in which a decreased expression was found. It is worthwhile mentioning, however, that IGF-I mRNA was found to be decreased in only 50% of kidneys in early diabetes, and was most prominent in the outer strip of outer medulla (Landau *et al.* 1995). Determination of IGF-I mRNA in the whole kidney tissue in a relatively early stage of DM as performed in our study could have failed to reveal more subtle variations in gene expression.

In our study, the lack of significant modifications in the IGF-I axis component gene expression following Oc therapy parallels the relative stability of renal function and renal mass. Actually, after the lowest dose of Oc, increased

creatinine clearance and more pronounced hyperglycemia, but no significant change in renal weight, were noted as compared with untreated DM or DM animals treated with higher Oc doses. After Oc administration in a very low dose insulin levels were also higher than those in untreated DM. Similar findings described previously in diabetic patients, in which plasma insulin was increased, were explained by a pharmacokinetic effect, i.e. a decreased selective blood flow with diminished access of insulin to its degradation sites (liver and kidney) and subsequently reduced metabolic clearance of insulin by somatostatin (Ipp *et al.* 1987, Kollind *et al.* 1990). These effects of Oc were not reproduced in our study by higher doses of the analog.

Our findings regarding the lack of effect of low dose Oc on renal function are similar to those reported by Muntzel *et al.* (1992) in their uninephrectomized diabetic rats treated with low dose Oc. In that study, Oc had no influence on renal hemodynamics and actually even increased the GFR value, when this parameter was normalized for kidney weight.

The apparent discrepancy between our findings and those showing a suppressive effect of Oc on renal function and growth in diabetes is not completely understood, and it may be related to the low dose used by us in contrast to the pharmacological doses used by other investigators, or to the duration of treatment. Furthermore, since the various actions of somatostatin and its analogs are mediated through five specific membrane receptors with unique tissue distributions, ligand binding properties and probably different signal transduction pathways (Lamberts *et al.* 1996, Patel *et al.* 1996), administration of Oc in various doses may be associated with different receptor binding patterns and with different physiological effects.

It is worthwhile mentioning that biphasic or apparently contradictory effects of somatostatin or its analogs have already been described in several tissue lines and clinical settings. Thus, in thyroid follicular cells, somatostatin in low doses inhibits basic and IGF-I-stimulated thyroid-stimulating hormone synthesis and cell proliferation, but has no effect when used in higher doses (Tsuzaki & Moses 1990). In cultured hepatocytes, Oc at low but not high concentrations inhibits IGF-I mRNA expression (Serri *et al.* 1992).

It is well known that patients with somatostatinoma and high plasma levels of somatostatin may present with a whole spectrum of disturbances in glucose metabolism, from mild hypoglycemia to overt diabetes and ketoacidosis, as a result of different kinds of imbalance between suppressory effects on insulin, glucose and GH secretion (Wilding *et al.* 1996).

One possible way to understand the biphasic effects of somatostatin and its analogs will require investigation of their recently described immunomodulatory properties. These effects which may interfere with the endocrine activities, include inhibition of locally induced mediators

like tumor necrosis factor (TNF) and nitric oxide (Karalis *et al.* 1994, Landa *et al.* 1995). In certain circumstances, the effect of somatostatin on the bioactivity and production of cytotoxins, especially TNF, was described to be paradoxical, i.e. enhancement of TNF activity by a lower concentration and suppressed activity in the presence of a higher concentration (Yousefi *et al.* 1991). Since TNF induces insulin resistance (Bazzoni & Beutler 1996), its modulation by various doses of somatostatin or analogs may lead to an aggravation or attenuation of a pre-existing diabetic state.

These questions, however, will require further confirmation by new and extensive laboratory studies.

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