Long-term effects of a somatostatin analogue on renal haemodynamics and hypertrophy in diabetic rats

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1. To determine whether treatment with octreotide, a somatostatin analogue, may diminish or prevent long-term diabetic renal hypertrophy and nephropathy, uninephrectomized streptozotocin-diabetic rats maintained under moderate glycaemic control (~300 mg/dl) were treated with either placebo (n = 10 rats/group) or octreotide for 14 weeks. Uninephrectomized non-diabetic rats given either placebo or octreotide served as controls.

2. Average body weight was diminished and kidney weight, daily urinary protein excretion, glomerular filtration rate and renal plasma flow were elevated in both diabetic groups relative to controls.

3. Administration of octreotide reduced average body weight and packed cell volume in non-diabetic and diabetic rats compared with their respective controls, but did not affect glomerular hyperfiltration or the increase in urinary protein excretion.

4. Histological examination at 14 weeks disclosed unequivocal glomerular hypertrophy and mild glomerular and tubulointerstitial lesions consistent with early diabetic renal alterations in all diabetic rats, but there was no independent effect of octreotide treatment.

5. Thus, long-term treatment with octreotide did not afford protection against the development of renal hypertrophy–hyperfiltration and the evolution of early diabetic nephropathy in rats.

INTRODUCTION

Diabetic glomerulopathy is the most serious complication of insulin-dependent diabetes. Nephropathy eventually develops in 30–40% of diabetic patients and is responsible for tenfold increases in cardiovascular mortality due to end-stage renal failure and hypertension [1, 2]. Although the mechanisms responsible for the development of diabetic nephropathy are ill-defined, glomerulosclerosis is preceded by a stage of renal hyperfiltration and hypertrophy [3, 4]. In streptozotocin-diabetic rats, glomerulosclerosis developed despite adequate glycaemic control, supporting a predominancy of renal haemodynamic alterations over metabolic control in the development of glomerulopathy [5–7].

More recently, Yoshida et al. [8], using a non-diabetic remnant kidney model, demonstrated that renal hypertrophy can induce glomerulosclerosis without concomitant glomerular hypertension. A role for growth factors in the induction of renal hypertrophy and glomerulosclerosis was also shown in studies using transgenic mice [9]. These animals expressing exaggerated levels of either insulin-like growth factor 1 (IGF-1) or growth hormone (GH), displayed glomerular and renal hypertrophy. In addition, transgenic mice expressing elevated levels of GH, but not of IGF-1, developed morphological lesions typical of diabetic glomerulosclerosis despite the absence of hypertension and hyperglycaemia. In diabetic patients characterized by poor metabolic control, increases in plasma GH concentration have been reported [10, 11], suggesting a stimulatory role of GH or its primary effector, IGF-1, in hypertrophic processes within the kidney and other vascular beds [10].

Octreotide, a somatostatin analogue, is a potent suppressor of GH secretion and has provided successful therapy in diseases characterized by excessive GH release, such as acromegaly. In acute studies examining diabetic rats, octreotide reduced diabetic hyperfiltration, apparently by a direct effect on vascular smooth muscle [12, 13]. Additional short-term studies showed that octreotide reduced and even prevented initial renal hypertrophy in streptozotocin-treated rats [14, 15]. Recently, Serri et al. [16] administered octreotide as a continuous subcutaneous infusion to humans with insulin-dependent diabetes for a period of 12 weeks. Compared with control diabetic patients, patients treated with octreotide exhibited significant reductions in glomerular filtration and mean total kidney volume at the end of the trial period.

Although these findings are encouraging, none has examined the preventative role of octreotide in the progression of diabetic glomerulosclerosis. We therefore assessed the effects of chronic octreotide therapy on the prevention of renal hypertrophy and
METHODS

We purchased 40 male Sprague-Dawley rats, aged 8 weeks, from Iffa Credo (L’Arbresle, France). The animals were housed in groups of three in a temperature-controlled colony room equipped with a 12 h light/12 h dark cycle. Throughout the experiment, the animals were given continuous access to tap water and a standard rat pellet diet containing 25% (w/v) protein.

After 1 week of habituation, all rats were subjected to right nephrectomy under ether anaesthesia. After 3 days of recovery, the rats were randomly divided into four groups (n = 10): a control group receiving daily injections of placebo, an octreotide group receiving daily subcutaneous injections of 20 μg of octreotide (Sandostatin; Sandoz, Basel, Switzerland), a diabetic group given a single intravenous injection of streptozotocin (60 mg/kg) in citrate buffer under light ether anaesthesia, followed by daily evening injections of a heat-treated ultralente insulin (Novo Industries, Copenhagen, Denmark), and a diabetic group treated with octreotide. Daily insulin doses were adjusted to maintain the blood glucose concentration between 200 and 400 mg/dl. These injections averaged approximately 2 units/day. The first octreotide injection of 20 μg was given 1 h after streptozotocin administration, and octreotide was injected thereafter as two daily injections of 10 μg each.

During the development stage of the experiment, lasting approximately 4 months, several parameters were regularly examined including blood glucose concentration and urinary protein excretion. Blood glucose concentration was determined every 4 weeks in tail blood from conscious rats, placed on bandelettes (Boehringer, Mannheim, Germany) and quantified by photometry (Boehringer). Monthly metabolic cage measurements provided estimations of 24 h food and water intakes as well as urinary protein excretion (Biotrol Protein Kit; Biotrol, Paris, France).

At the completion of the developmental phase, all rats underwent haemodynamic studies. One hour before surgery, diabetic and non-diabetic rats assigned to octreotide treatment were administered a final 20 μg injection of octreotide, whereas controls received placebo. All rats were anaesthetized with pentobarbital (50 mg/100 g body weight) and placed on a warming table regulated at 38°C. Polyethylene catheters (Clay Adams, Parsippany, NJ, U.S.A.) were inserted in the trachea, right jugular vein, right and left femoral arteries and in the urinary bladder. Saline solution (150 mmol/l) was administered via the jugular catheter at a dose of 0.5% of body weight over 20 min to replace plasma losses associated with anaesthesia and surgery. Infusion was continued using a solution of [3H]inulin (6.5 μCi/ml), p-[14C]aminohippurate ([14C]PAH) (0.6 μCi/ml), pentobarbital (2.5 mg/ml) and saline given at a rate of 10 μl min⁻¹ 100 g⁻¹ body weight. After an equilibration period of 1 h, urine was collected from the bladder catheter during three 30 min periods. Blood was drawn continuously from the right femoral catheter using an infusion pump (model 351; Sage Instruments, Cambridge, MA, U.S.A.) at a rate of 13 μl min⁻¹ 100 g⁻¹ body weight, to provide a total of 390 μl of blood for each period. Packed cell volume (PCV) was immediately recorded. Systolic blood pressure was recorded throughout the clearance periods via the left femoral catheter using a transducer (P23DB; Gould-Statham, Inc. Cleveland, OH, U.S.A.) and an amplifier with a recorder (Gould Instruments, Ballainvilliers, France). Radioactivity was measured using a liquid scintillation counter (1212 Rackbeta; LKB Wallac, Turku, Finland).

Clearances of inulin (C₀) and PAH (C_PAH) were calculated by standard methods. Filtration fraction (FF) was calculated from C₀/C_PAH, and arterial blood pressure. At the end of the clearance studies, blood was collected from the descending aorta into tubes containing EDTA, centrifuged, and plasma samples were stored at −80°C for later analysis.

Plasma IGF-1 concentration was assayed after a modified acid ethanol extraction using a cyro-precipitation step [18]. R.I.a. was performed using rabbit anti-human IGF-1 antibody, UBK-487, obtained from NTBP (Baltimore, MD, U.S.A.), radiolabelled 125I-IGF-1 (Amersham, Cardiff, U.K.), and recombinant IGF-1 as a standard (ARN 4140, Amersham). Separation of bound and free fractions was achieved with PR 1000, an immunoprecipitating reagent (iCIS, Gif-sur-Yvette, France). Cross-reaction with rat IGF-1 was found to be more than 90%. All samples were measured at one time giving an intra-assay coefficient of variation of 7.5% at a concentration of 150 ng of IGF-1/ml.

Immediately after exsanguination, each kidney was removed, carefully cleaned, weighed, and two coronal slices 2–3 mm thick were fixed in Dubosq Brasil fixative. Three-micrometre thick paraffin sections were then stained using Mason’s trichrome with Light Green, periodic acid–Schiff and silver impregnation according to the methods of Jones, respectively, for examination by light microscopy. The sections were coded and analysed by a pathologist (L.-H.N.) in a double-blind manner. For each specimen, a mean of 100 glomeruli were examined from comparable portions of the kidney, midway between the two poles. A semi-quantitative system
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![Graph showing progression of body weight in uninephrectomized (UNX) rats during 14 weeks of study. The experimental groups (n=10 rats/group) were: control rats (●●●), octreotide-treated rats (●●●●●), diabetic rats (○○○○○) and octreotide-treated diabetic rats (○○○○○). The rate of increase of body weight was diminished by diabetes and by treatment with octreotide. Abbreviation: STZ, streptozotocin.](image)

![Graph showing blood glucose concentration in control rats (●●●), octreotide-treated rats (●●●●●), diabetic rats (○○○○○) and octreotide-treated diabetic rats (○○○○○). A moderate hyperglycaemia was maintained in the diabetic groups throughout the study.](image)

was used to grade glomerular and tubular pathology consistent with diabetic alterations. Mild interstitial lesions were defined by the presence of tubular dilatations and cysts. Severe tubular lesions were defined by the presence of focal fibrosis, inflammatory cells, tubular dilatation and/or intratubular casts. Glomerular lesions were characterized by mesangial expansion with focal and/or segmental glomerulosclerosis.

The data are presented as means ± SEM, and were analysed using appropriate single or repeated measures two-way analysis of variance [19]. Prevalence of pathological lesions in the different groups were examined using a contingency table (χ² test). Differences between groups were considered significant at the 0.05 level.

RESULTS

Long-term outcomes

The evolution of body weight, over 14 weeks of study, is illustrated in Fig. 1. Although all rats gained weight during the course of the study (P < 0.0001), diabetes suppressed normal growth, resulting in differences of 100 g between diabetic and control groups at the completion of the trial (P < 0.0001) independently of this effect, octreotide diminished growth rate equally in diabetic and non-diabetic groups (P < 0.001).

As shown in Fig. 2, diabetic rats exhibited moderate hyperglycaemia during the entire study (P < 0.0001). Hyperglycaemia was relatively stable, except for a decline during week 6 of the study due to adjustments in insulin administration (P < 0.0001). That diabetes induced degenerative changes in renal function was indicated by progressive increases in urinary protein excretion in both diabetic groups (Fig. 3). Elevated protein excretion was manifested during the second month of diabetes (P < 0.05), and increased exponentially thereafter.

Kidney weight, metabolic status and indications of extracellular fluid balance at the completion of the study are shown in Table 1. Diabetes generated an expected increase in kidney size (P < 0.0001), although no effect of octreotide was observed. Rats made diabetic also exhibited hyperphagia (P < 0.001) and polydipsia (P < 0.001) compared with controls. PCV was slightly reduced by diabetes (P < 0.05) and by octreotide treatment (P < 0.05). Similar findings were noted for plasma protein concentrations, although these alterations did not attain statistical significance. Induction of diabetes profoundly depressed serum IGF-1 levels (P < 0.0001) with no effect of octreotide treatment.

Haemodynamic studies

Diabetic rats exhibited a pattern of renal haemodynamics characteristic of animals in moderate glycaemic control (Table 2). The analysis of variance for systolic blood pressure in anaesthetized rats revealed a condition by treatment interaction.
Both diabetic groups exhibited a moderate rise in urinary protein excretion rate near the end of the study. (Pc0.05) reflecting higher blood pressures in octreotide-treated non-diabetic rats relative to their placebo-treated counterparts. Whole-kidney GFR was elevated in both diabetic groups in relation to control rats (P<0.01). However, when GFR was normalized for kidney weight hyperfiltration in diabetic animals was abolished. In addition, the tendency for elevated GFR in the two octreotide-treated groups became significant when GFR was normalized for kidney weight (Pc0.05). Diabetic rats treated with octreotide and placebo exhibited modest elevations in renal plasma flow (P<O.O5). FF was also higher in diabetic groups (P<0.01) and RVR tended to be lower, although this difference was not significant.

**Light microscopy**

The main consistent pathological feature was a striking glomerular enlargement in diabetic rats, which was independent of treatment with octreotide or placebo (Figs. 4a–4c). Renal pathological lesions were otherwise relatively scarce and totally absent in both of the diabetic groups. In kidneys with glomerular lesions, no more than 10% of glomerular were affected. For this reason, lesions were expressed not as incidence of glomerular lesions, but as incidence of kidneys demonstrating significant tubular or glomerular lesions (Table 3). Tubular lesions were significantly more frequent in the diabetic groups (P<0.01) than in the control groups, but no effect of octreotide was found in either the non-diabetic or diabetic groups. Glomerular lesions were significantly more frequent in diabetic groups than in non-diabetic groups (P<0.05) and in octreotide-treated groups than in their respective placebo-treated groups (P<0.05) irrespective of diabetes status.

**DISCUSSION**

In agreement with earlier reports [3, 4, 7], insulin-dependent diabetic rats maintained under moderate glycaemic control were characterized by renal hypertrophy, glomerular hyperfiltration and subnormal body growth despite hyperphagia. The causes of hyperfiltration appeared to be multifactorial. Elevated glomerular filtration appeared to be related to reductions in RVR associated with increased renal plasma flow [7]. However, increased FF in diabetic rats suggested either an increase in glomerular pressure gradient or an increase in ultrafiltration coefficient [3, 17] through glomerular hypertrophy and presumably increased filtration surface area. The preponderant influence of renal hypertrophy on hyperfiltration was inferred in the current study since GFRs in diabetic rats were abolished when the parameter was normalized for kidney weight [3, 17].

That serum IGF-1 levels are markedly reduced in streptozotocin-diabetic rats has been known for some time [20]. The reduced serum IGF-1 levels in our diabetic rats may explain, in part, the reduced growth rates of the two diabetic groups [21]. Octreotide was further reported to decrease renal tissue IGF-1 concentrations in the short term [14, 15]. However, the relative contribution of tissue and circulating IGF-1 to bioactivity is not known. Renal tissue IGF-1 concentration increases rapidly with the onset of diabetes in rats and parallels the development of renal hypertrophy [14, 22]. Paradoxically, renal IGF-1 messenger RNA, a marker of cellular gene expression for the hormone, is unaltered and even depressed in diabetic rats [20, 23]. Although enhanced translation of messenger RNA cannot be excluded, the elevated tissue IGF-1 level may simply reflect enhanced uptake of plasma IGF-1 by the diabetic kidney.

Serum GH levels are increased in diabetic patients characterized by poor metabolic control, but are diminished in streptozotocin-diabetic rats [24]. However, when dwarf rats, which are genetically deficient in GH, were rendered diabetic by streptozotocin, renal hypertrophy was blunted despite a significant increase in the renal IGF-1 level [25]. These findings suggest that a synergistic interaction between circulating GH and IGF-1 has an effect on the full expression of renal hypertrophy in diabetes. Although it may have been valuable to characterize plasma GH in the current study, plasma levels of this hormone undulate widely in
rats as a result of pulsatile secretion [26]. Thus, several GH determinations would be necessary to obtain integrated values, requiring an analysis beyond the scope of this study.

In our model of diabetes, unilateral nephrectomy was included to accelerate glomerular injury. We intended to reproduce the findings of O’Donnell et al. [17], who observed severe glomerulosclerotic changes after 3 months of diabetes in young uninephrectomized Sprague-Dawley rats.

In contrast to their findings of significant mesangial matrix expansion and focal glomerulosclerosis, rats with poor metabolic control may have consumed greater amounts of food resulting in greater protein intakes, an effect known to accelerate glomerulosclerosis [27]. Since renal tissue lesions in our diabetic model were relatively scarce, it was difficult to establish a preventative effect of long-term octreotide treatment. Nevertheless, the degree of glomerular hypertrophy and the incidence of tubular dilatation and glomerular segmental lesions were at least similar in diabetic rats treated by octreotide or by placebo.

Only a single published study has described longer-term effects of octreotide in diabetes. Serri et al. [16] demonstrated that 12 weeks of subcutaneous oteotride administration reduced GFR and kidney volume in patients with insulin-dependent diabetes. In the light of these findings, the lack of salutary effects of octreotide in diabetic rats is puzzling. The ability of the drug to influence diabetic pathology may depend on several factors including dose, the presence of pre-existing diabetes and on differing responses to octreotide between humans and rats [7].
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Table 3. Renal tissue pathology after 14 weeks of diabetes

<table>
<thead>
<tr>
<th>Group</th>
<th>Mild tubular lesions (%)</th>
<th>Severe tubular lesions (%)</th>
<th>Glomerular lesions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo, non-diabetic</td>
<td>50.0</td>
<td>16.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Octreotide, non-diabetic</td>
<td>50.0</td>
<td>0.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Placebo, diabetic</td>
<td>33.3</td>
<td>41.7</td>
<td>25.0</td>
</tr>
<tr>
<td>Octreotide, diabetic</td>
<td>15.4</td>
<td>38.5</td>
<td>46.2</td>
</tr>
</tbody>
</table>

In fact, the high doses in the present study produced an unexpected attenuation of body growth in both diabetic and non-diabetic octreotide-treated groups, a finding previously unreported in the literature on diabetes. Drug-induced sickness or inactivity cannot explain this result since food and water intakes were similar in octreotide and placebo-treated groups.

Alternatively, the absence of effects of octreotide may be related to good metabolic control in diabetic rats in the current study. Indeed, strict insulin treatment abolishes increases in both kidney IGF-1 levels and renal hypertrophy.

Furthermore, the degree of kidney IGF-1 accumulation 2 days after streptozotocin administration was found to be directly proportional to blood glucose levels in rats with graded severities of diabetes [30]. The combination of diabetes and uninephrectomy induces additive increases in both kidney IGF-1 accumulation and renal enlargement [31], suggesting that some of these hypertrophic stimuli could be unresponsive to octreotide.

In summary, long-term octreotide therapy did not prevent early pathological changes characteristic of insulin-dependent diabetes in rats. Although the current dose of octreotide was strong enough to inhibit normal body growth and reduce PCV, the drug did not inhibit diabetic renal hypertrophy, glomerular hyperfiltration, tissue damage or the development of elevated urinary protein excretion after 14 weeks of diabetes.

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REFERENCES

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