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Somatostatin analogue administration prevents increase in kidney somatomedin C and initial renal growth in diabetic and uninephrectomized rats

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Summary. In a previous study we demonstrated that the kidney content of somatomedin C was maximal one to two days after uninephrectomy or induction of diabetes, and that insulin treatment prevented an increase in kidney somatomedin C as well as kidney growth in diabetic animals. In the present study we ave examined the effect of a somatostatin analogue on kidney somatomedin C and initial renal growth in the two experimental situations. The kidney hypertrophy in untreated diabetic animals amounted to 23% four days after streptozotocin injection and followed an increase in kidney somatomedin C content of 60% reaching the maximum after 48 h. In young and old uninephrectomized rats kidney growth was 19% and 16% after four days. In young animals a prompt increase of 50% in kidney somatomedin C was seen as reaching the maximum after 24 h, while the somatomedin C content in kidneys from old animals was maximal after 48 h (increase of 58%) in good accordance with the slightly slower kidney growth. The new findings of the present study are that administration of a long-acting somatostatin analogue (Sandostatin) effectively prevented the obligatory increase in kidney somatomedin C content as well as kidney growth both in experimental diabetes and after uninephrectomy. It is noteworthy that Sandostatin administration did not alter the metabolic state in diabetic animals indicating that the inhibition of kidney hypertrophy could not be attributed to improved metabolic control. The results thus support the concept that somatomedin C is involved in initial diabetic and post-nephrectomy renal growth.

Key words: Diabetic nephropathy, hypertrophy, kidney, nephrectomy, rat, somatomedin C, somatostatin-analogue, streptozotocin diabetes.

Kidney growth in diabetes of recent onset has been compared to kidney growth following unilateral nephrectomy [1, 2]. The kidney growth has been extensively studied and possible explanations have included involvement of various humoral factors [1, 3, 4]

A role for the growth factor somatomedin C (SMC) has been proposed in recent biochemical studies [2, 5, 6] and in one immunohistochemical study [7].

It has recently been demonstrated that somatostatin infusion suppresses hyperfiltration and renal plasma flow in Type 1 (insulin-dependent) diabetic patients apparently by a direct effect [8, 9] and that a long-acting somatostatin analogue reduces renal growth in alloxan diabetic rats [10].

Bearing in mind the wide suppressive action of somatostatin on hormones, we investigated the effects of a somatostatin analogue on the kidney SMC accummulation in diabetic and uninephrectomized rats.

Materials and methods

Male Wistar rats (Møllegaards Avlslab., Eiby, Denmark) were used in all studies. Diabetes was induced by intravenous injection of streptozotocin (STZ) (pH = 4.0) in a dose of 55 mg/kg body weight and in the studies dealing with post-nephrectomy renal growth, left-sided nephrectomy was performed under sodium barbital anaesthesia. In the diabetic animals blood glucose was measured daily (Haemo-Glucotest 1-44 and Reflolux II, Boehringer-Mannheim, Mannheim, FRG) and the urine was tested for glucose and ketone bodies by Neostix-4 (Ames, Stoke Poges, Slough, UK). All the animals were weighed every day. Blood (300 µl) was taken from the retrobulbar venous plexus through heparinized capillary tubes, centrifuged and serum frozen at −20° C for later analysis. The rats lived 3 to 5 in cages in a room with a 12:12 h (06.00-18.00 hour) artificial light cycle, temperature 21 ± 2° C and humidity 55 ± 2%, with free access to food and water.

Protocol

The following four groups were studied: 1) diabetic rats (body weight: 206-246 g); 2) young, uninephrectomized rats (body weight: 195-234 g); 3) old, uninephrectomized rats (body weight: 370-480 g); and 4) control rats (body weight: 202-240 g).

In each group half of the animals were treated with Sandostatin (Sandoz, Ltd., Basel, Switzerland) by SC injections. The other half received an equivalent volume of 0.9% NaCl. The treatment by long-acting synthetic somatostatin analogue was started immediately after STZ injection or unilateral nephrectomy. A Sandostatin dose large enough to maintain high diurnal serum concentrations (above 1000 ng/l) was used, i.e. 100 µg twice daily. The (right) kidneys removed during the experimental period were trimmed of fat, hilus and capsule, weighed and immediately frozen in liquid nitrogen.

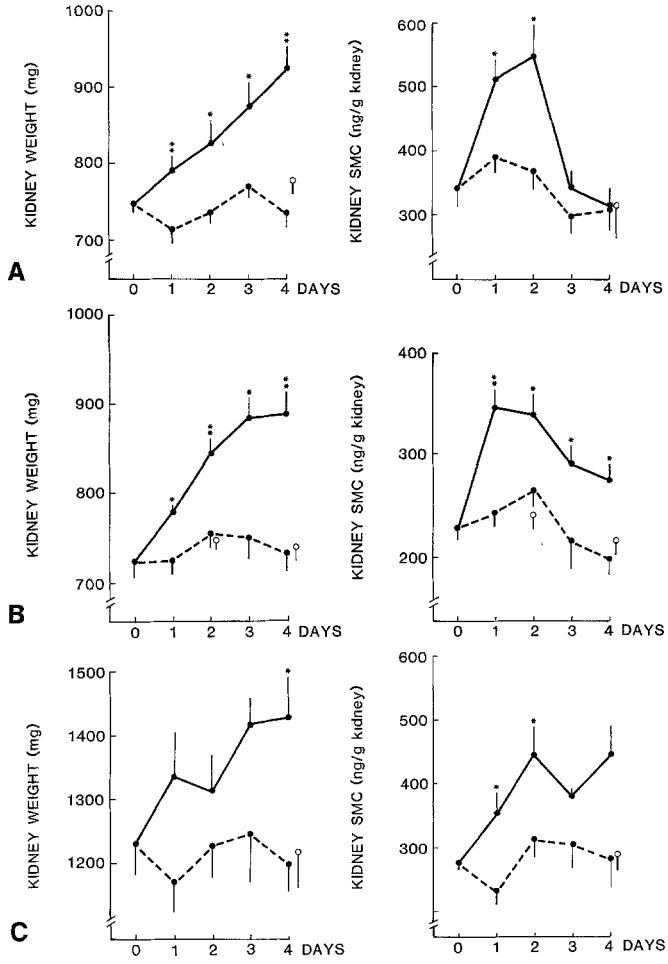

Fig. 1 A-C. Changes over 4 days in kidney weight (left) and kidney somatomedin C (SMC) (right) in **A:** streptozotocin diabetic rats, untreated (●——●), Sandostatin treated (●----●) and non-diabetic controls (○); **B:** young and **C:** old unilaterally nephrectomized rats, untreated (●——●), Sandostatin treated (●----●) and sham operated rats (○) * $p<0.05$, ** $p<0.01$ between untreated and Sandostatin treated diabetic and nephrectomized rats and compared to non-diabetic control and sham operated rats. Mean ± SEM, $n=5$ for all studies except the diabetic study where $n=6$

In the first study dealing with diabetic rats, two groups ($n=6$), with and without Sandostatin, were taken out each day for the following four days after STZ injection and their kidneys were removed under sodium barbital anaesthesia. Non-diabetic control rats were taken out on day 0 and day 4. Similarly, in the uninephrectomized animals, during the second and third study, two groups ($n=5$), both with and without Sandostatin administration, had right sided nephrectomies performed each day for the following four days. Normal rats at day 0, and sham operated rats at day 2 and day 4 served as controls. In the fourth study normal rats receiving Sandostatin underwent similar treatment, with two groups ($n=5$) taken out each day.

Somatomedin C extraction from kidney

SMC extraction was performed according to D'Ercole [11] as previously described [2]. Briefly, the frozen kidney was homogenized on ice in 1 mol/l acetic acid (5 ml/g tissue) with an Ultra Turrax TD 25 and further disrupted using a Potter Elvehjelm homogenizer. The extract was incubated on ice for 2 h, centrifuged at 4000 rev/min for 15 min and the supernatants decanted. The pellet was re-extracted once, the supernatants were pooled and lyophilized to dryness.

The sample was redissolved in 40 mmol/l phosphate buffer, pH 8.0, at a ratio of 5 ml buffer/g tissue weight. Tissue extracts were kept at −20°C until SMC assay was performed.

Somatomedin C radioimmunoassay

SMC was estimated as described previously [2] using SMC antibody UB 286 (raised by L.E. Underwood and J.J. van Wyk, Paediatric Endocrinology, University of North Carolina, Chapel Hill, NC, USA) donated by the National Hormone and Pituitary Program.

SMC immunoactivity was measured in diluted rat serum (1:400) after previous extraction in acetic-methanol [2].

Sandostatin radioimmunoassay

Sandostatin, antibody and ^{125}I-labelled antigen was donated by Drs. A.G. Harris and J. Rosenthaler (Sandoz, Ltd., Basel, Switzerland) and used as previously described [12].

Statistical analysis

Statistics were performed using one-way analysis of variance (ANOVA) combined with the Bonferroni test for multiple comparisons and unpaired Student's t-test. Significant differences between untreated and Sandostatin treated rats are shown in the figures, while the significance of changes within groups from day 0 level are given in the text.

Results

Kidney growth

As shown in Figure 1 (left) the diabetic (A) and young uninephrectomized rats (B) sustained a rapid increase in wet kidney weight, while a relatively slower growth was seen in the old uninephrectomized rats (C). Four days after STZ injection the increase from baseline was 23% in diabetic rats (748 ± 9 mg (SEM) to 919 ± 27 mg, $p<0.01$) while increases of 19% and 16% were seen after four days in young and old uninephrectomized rats (737 ± 26 mg to 880 ± 29 mg, $p=0.02$ and 1230 ± 45 mg to 1429 ± 67 mg, $p=0.01$, respectively). In the normal control rats no change was observed in kidney weight during the four days. As illustrated in Figure 1, left (A, B, C) the administration of Sandostatin to diabetic and uninephrectomized animals effectively prevented kidney growth, with no differences from the non-diabetic or sham-operated control animals, respectively. Sandostatin administration had no effect on kidney weight in normal animals (data not shown).

It is noteworthy that no significant alteration in blood glucose was seen in response to Sandostatin administration, illustrated in Figure 2 for the diabetic animals.

Kidney somatomedin C

The change in kidney content of SMC is illustrated in Figure 1 (right). In the diabetic animals (A) a prompt increase of 60% from baseline was seen, reaching a

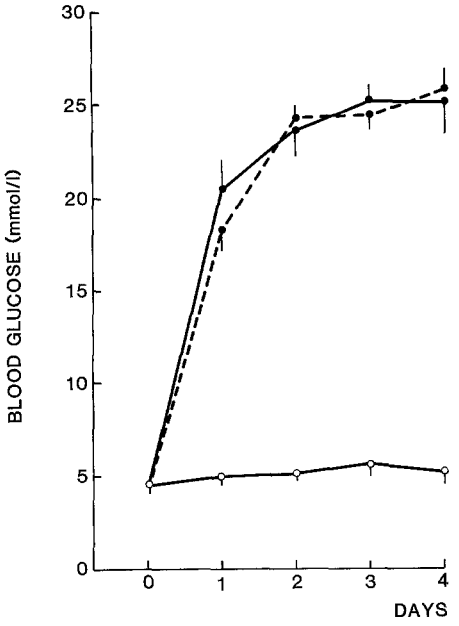

Fig. 2. Changes in blood glucose in streptozotocin diabetic rats, untreated (●——●) and Sandostatin treated (●----●), over 4 days. Streptozotocin injected day 0 at a dose of 55 mg/kg i.v. Non-diabetic control rats (○——○). Mean ± SEM. Day 0, $n=60$. Diabetic groups: day 1, $n=24$; day 2, $n=18$; day 3, $n=12$; day 4, $n=6$. In non-diabetic controls each point represents 6 different animals

maximum after 48 h (340 ± 28 ng/g (SEM) to 544 ± 55 ng/g, $p<0.01$). Over the following two days SMC content returned to the initial level. The diabetic animals treated with Sandostatin showed no significant change in kidney SMC during the observation period. In uninephrectomized rats the SMC content in the remaining kidneys was maximal after 24 h in young rats (B), with an increase of 50% from day 0 (from 227 ± 8 ng/g to 342 ± 18 ng/g, $p<0.01$), and four days after nephrectomy the kidney SMC was still significantly elevated ($p=0.01$). Treatment with Sandostatin effectively prevented the increase in kidney SMC. In old uninephrectomized rats kidney SMC was maximal after 48 h with an increase of 58% (279 ± 8 ng/g to 442 ± 43 ng/g, $p<0.01$) and again andostatin inhibited accumulation of kidney SMC. In the normal rats no changes were found in kidney SMC either with or without Sandostatin treatment (data not shown).

Serum somatomedin C

The untreated diabetic animals showed a 33% decrease from baseline in circulating SMC after four days, while the Sandostatin treated diabetic rats had even greater reduction of serum SMC, reaching 63% of day 0 levels (Fig. 3 A, left).

Young uninephrectomized animals had a 14% decrease in serum SMC four days after surgery when compared to sham operated ones ($p=0.02$), while old uninephrectomized rats had a decrease 24 h after

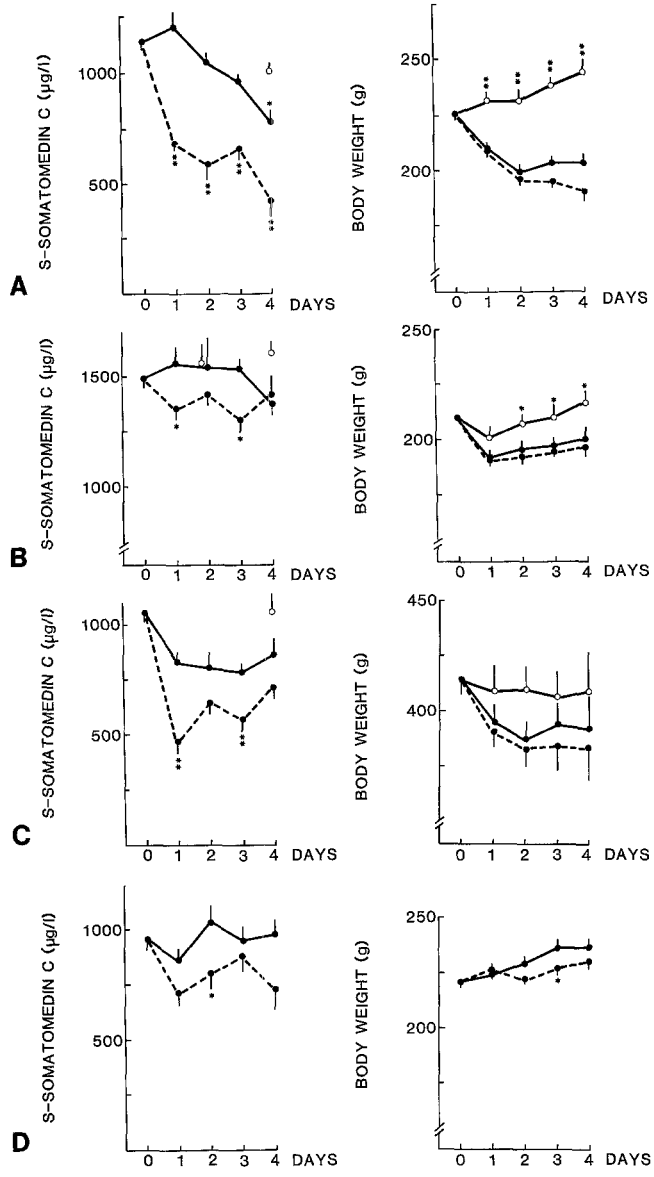

Fig. 3 A–D. Changes over 4 days in serum somatomedin C (left) and body weight (right) in **A:** streptozotocin diabetic rats, untreated (●——●), Sandostatin treated (●----●) and non-diabetic control rats (○——○); **B:** young and **C:** old unilaterally nephrectomized rats, untreated (●——●), Sandostatin treated (●----●) and sham operated control rats (○——○); **D:** normal control rats, untreated (●——●) and Sandostatin treated (●----●). * $p<0.05$, ** $p<0.01$ between untreated and Sandostatin treated diabetic and nephrectomized rats and compared to non-diabetic control and sham operated rats. Mean ± SEM. For serum SMC, day 0 values represent all animals i.e. **A:** $n=60$, **B:** $n=55$, **C:** $n=50$ and **D:** $n=45$, while each point the following days represents $n=6$ in **A** and $n=5$ in **B–D.** Body weight values include all animals day 0 and from day 1 to 4 each point represents the remaining animals i.e. $n=24, 18, 12, 6$ in the diabetic study, and $n=20, 15, 10, 5$ in the nephrectomy and normal control studies. Non-diabetic control rats ($n=6$) and sham operated animals ($n=5$)

surgery amounting to 22% ($p<0.01$) and remained at this lower level throughout the observation period (Fig. 3 B, C (left)). Sandostatin treated groups had lower serum SMC than the untreated counterparts throughout the course of the experiment. Figure 3 D

(left) shows the slight decrease in serum SMC in normal control rats treated with Sandostatin, significantly lower only at day 2 compared to the untreated controls (1044 ± 64 µg/l (SEM) vs 800 ± 60 µg/l, $p = 0.025$).

Body weight

Both diabetic groups had a significant 13% ($p < 0.01$) decrease in body weight two days after STZ (Fig. 3 A (right)) and there was no difference in body weight between Sandostatin and untreated diabetic animals during the observation period. Young, uninephrectomized rats had a significant 10% weight loss 24 h after surgery ($p < 0.01$) with an insignificant weight gain over the following three days (Fig. 3 B (right)). No difference was found between the Sandostatin and the untreated groups. Old, uninephrectomized animals had a significant weight loss two days after surgery of 7% ($p = 0.02$), no difference was found between treated and untreated groups (Fig. 3 C (right)). In the normal rats receiving Sandostatin no difference in body weight, except on day 3 (227 ± 5 g (SEM) vs 238 ± 3, $p < 0.01$), was noted compared to the untreated controls (Fig. 3 D (right)).

Discussion

The present study confirms the findings in a recent paper that initial renal growth in diabetic and uninephrectomized rats follows an increase in kidney SMC content [2]. The new findings are that administration of a long-acting somatostatin analogue (Sandostatin) prevents the increase in kidney SMC and kidney growth in diabetic and uninephrectomized rats. Furthermore, it is demonstrated that the increase in kidney SMC in response to uninephrectomy peaks somewhat later in old rats than in young ones, being in accordance with the somewhat slower percentual increase in kidney weight in old animals.

These results reinforce the proposal that SMC may have a central role in initial renal growth in both diabetic and uninephrectomized rats.

The experimental conditions were optimal for studies of initial diabetic renal hypertrophy, i.e. blood glucose concentrations around 25 mmol/l, no ketonuria and minimal body weight loss [1]. The changes in kidney weight in both diabetic and uninephrectomized animals are consistent with earlier findings [1, 2] and the fact that strict insulin treatment prevents both diabetic kidney growth and the increase in kidney somatomedin C [2] indicates that these changes are not caused by streptozotocin per se.

A few studies have appeared dealing with the effect of somatostatin and its synthetic analogues on *kidney function*. Somatostatin and Sandostatin infusion in Type 1 diabetic patients immediately suppresses hyperfiltration and renal plasma flow, apparently by a direct action on vascular smooth muscle [8, 9]. Furthermore, the administration of Sandostatin to alloxan diabetic rats has been shown to reduce initial *renal hypertrophy* [10], but no measurements of growth factors were performed in this latter paper.

In the present study the initial kidney growth in diabetic and uninephrectomized rats was prevented, while Steer et al. [10] found only a relative inhibition of renal growth in diabetic rats. This difference is probably due to the smaller Sandostatin dose used.

Administration of native somatostatin or Sandostatin has resulted in decreased fasting *blood glucose* and a reduction in postprandial hyperglycaemia in Type 1 diabetic patients [13]. Our results in rats, however, being in accordance with the findings of Steer et al. [10], showed no effects on blood glucose in Sandostatin treated diabetic rats. The tendency towards differences in body weight between Sandostatin and untreated diabetic animals after four days in the present study is, however, possibly related to the more pronounced decrease in serum SMC in the former.

Compensatory kidney growth after unilateral nephrectomy seems favourable from a physiological point of view, although it has been reported that glomerular sclerosis may develop in the remaining kidney after nephrectomy [14, 15].

Renal hypertrophy and hyperfunction in diabetes have been suggested as initiating or accelerating late diabetic nephropathy [16, 17], although the exact mechanism is unknown. The possible role of growth hormone in relation to diabetic nephropathy remains controversial [18–20]. However, in diabetic rats growth hormone secretion is inhibited [21, 22] rather than paradoxically stimulated as in diabetes in man.

The precise mechanism of the effects of Sandostatin on kidney SMC and kidney growth remains unsolved. The effects may well, at least in uninephrectomized animals, be mediated through a suppression of growth hormone secretion, since postnephrectomy renal growth in parts depends on the presence of an intact pituitary [5, 23]. Although our present findings are no proof of a direct effect of Sandostatin on kidney level, the lack of increase in kidney tissue SMC in diabetic rats during Sandostatin administration seems to point to a direct effect on local production, since growth hormone secretion is already reduced in diabetic rats as mentioned above [21, 22]. However, *serum* SMC is also reduced during Sandostatin administration and a direct inhibitory effect on hepatic SMC formation may also participate in the reduced SMC accumulation in the kidney.

The question of whether the SMC accumulation in hypertrophying kidneys is due to local synthesis or to increased uptake of circulating SMC and whether the Sandostatin effect is principally local or hepatic could probably be solved through perfusion studies of isolated kidney tissue and in studies with determination of kidney IGF-I mRNA levels.

In conclusion, the fact that metabolic control is not improved in diabetic animals receiving Sandostatin, taken together with the totally absent increase in kidney SMC and kidney growth, would strongly suggest a role of SMC in initial diabetic renal hypertrophy. In addition, the present study gives further evidence for the role of SMC in *compensatory* renal growth, since the increase in kidney SMC as well as the kidney growth is also effectively prevented by Sandostatin.

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Addendum

With regret we have to state that in our previous publication (Ref. 2) in this journal we made the mistake of twice correcting a dilution factor. This implies that the kidney SMC values of that paper must be divided by five to give correct values comparable with the present levels.