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Kidney tissue somatomedin C and initial renal growth in diabetic and uninephrectomised rats

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Summary. Kidney growth after induction of experimental diabetes in rats was compared to compensatory renal growth in response to unilateral nephrectomy. After 4 days of diabetes, kidney weight had increased from 816 ± 21 mg (SEM) to 940 ± 42 mg (15%). In insulin-treated diabetic rats kidney weight was unchanged at the end of the study, namely 828 ± 15 mg.

In unilaterally nephrectomised rats kidney weight increased from 840 ± 20 mg (SEM) to 1050 ± 60 mg during 4 days (24%). We observed increased kidney content of somatomedin C in both diabetic and uninephrectomised rats. In untreated diabetic rats it was maximal after 48 h, with an increase of 77% (3469 ± 312 ng/g (SEM) versus 1961 ± 173 ng/g). After 4 days the somatomedin C content had returned to initial levels. In insulin-treated rats somatomedin C content did not increase during the observation period. The somatomedin C content of the remaining kidney after unilateral nephrectomy was maximal after 24 h with an increase of 58% (from 1340 ± 203 ng/g (SEM) to 2122 ± 214 ng/g). The somatomedin C content returned to normal at day 4. Serum somatomedin C declined insignificantly in diabetic animals during the experimental period, but a significant decrease ($p < 0.02$) was found in uninephrectomised rats. This study demonstrates that kidney somatomedin C peaks during the first or second day after uninephrectomy or induction of diabetes, respectively, and that insulin treatment sufficient to prevent kidney growth abolishes the increase. These similar rapid initial hypertrophies/hyperplasies may thus be dependent on local somatomedin C formation.

Key words: Kidney, hypertrophy, nephrectomy, rat, somatomedin C, streptozotocin, diabetes.

It is well-known that kidney size and function are increased in diabetes mellitus of recent onset, as demonstrated in man [1, 2] and in experimental diabetic rats [3]. Kidney size and kidney function remain normal if strict metabolic control is attained immediately [3], or return towards normal when intensive insulin treatment is started early [4].

The mechanism responsible for renal growth and hyperfunction in diabetes is unknown. Several factors have been proposed as being solitary or concurrent causes [5, 6].

Kidney growth following unilateral nephrectomy has been compared to diabetic renal growth, and in many ways the two types of kidney growth are similar, although the initial growth may follow somewhat different cellular reactions [5].

Somatomedin C (SMC) is a polypeptide with potent mitogenic activity in vitro [7, 8]. In vivo, exogenous SMC stimulates growth in hypophysectomised rats [9]. Somatomedins are synthesised at multiple sites and their actions are exerted both distant from [10, 11] and at or near the sites of production [12, 13].

Kidney growth following diabetes of recent onset or uninephrectomy is manifest within 24-48 h. We measured serum and kidney tissue concentrations of SMC each day during the first 4 days after induction of diabetes or unilateral nephrectomy.

Materials and methods

Two studies were performed: one dealing with streptozotocin diabetic rats and the second with the effects of unilateral nephrectomy.

In the first study male Wistar rats (220-256 g) were used, purchased from Møllegaards Avlslab. (Eiby, DK).

The rats were kept five in each separate cage in a room with a 12:12 h artificial light cycle (06.00-18.00 hours), temperature 21 ± 2 °C and humidity 55 ± 2%) with free access to food and water.

Streptozotocin was given i.p. in a dose of 65 mg/kg body weight at day 0. Animals with a moderate degree of diabetes were produced, having blood glucose exceeding 15 mmol/l from day 1, but there was a minimal loss of body weight in the experimental period, i.e. less than 20 g.

At day 1 the diabetic animals were separated into two groups. One of these was treated with insulin s.c. every morning. A very long-acting heat-treated non-commercial ultralente insulin (NOVO,

Bagsvaerd, DK) was used, administered after blood glucose determination.

Under sodium barbital anaesthesia (50 mg/kg body weight) five diabetic animals had their right kidneys removed at day 0 and day 1. The following 3 days five rats of each group, i.e. insulin and non-insulin treated animals, had their right kidneys removed.

The kidneys were trimmed of fat and capsule, weighed and cut into two parts, one for SMC determination and one for measuring content of DNA, RNA and protein. After weighing, the samples were immediately frozen in liquid nitrogen.

Blood was sampled (500 µl) at day 0 in all rats and from the animals taken out each day for investigation. The samples were taken from the retrobulbar venous plexus through a heparinised capillary tube. Blood was centrifuged and serum frozen for later analysis.

Blood glucose was measured daily (Haemo-Glucotest 1-44 and Reflolux II, Boehringer-Mannheim, Mannheim, FRG). Urine was tested for glucose and ketone bodies every day with Neostix-4 (Ames, Stotze Poges, Slough, UK). The animals were weighed every day.

In the second study 30 male Wistar rats (210 298 g) were used, randomised into 6 groups. Under sodium barbital anaesthesia one group underwent bilateral nephrectomy and one group was sham-operated by flank incision and gentle manipulation of left kidney.

The last four groups underwent left uninephrectomy on day 0; and on each of the following 4 days five rats had their remaining kidneys removed, which was treated as for the diabetic rats.

Somatomedin C extraction from kidney

SMC extraction was performed as described by D'Ecole [12]. The frozen kidney was pulverized in 1 mol/l acetic acid (5 ml/g tissue) on ice bath. With this procedure pH ranges from 3.6-4.2 resulting in maximal liberation of SMC from tissue. The extract was incubated on ice for 2 h, centrifuged 4000 rpm for 15 min and the supernatant decanted. The pellet was reextracted once, the supernatants pooled and then lyophilised to dryness.

The sample was redissolved in 40 mmol/l phosphate buffer, pH 8.0, at a ratio of 10 ml buffer/g tissue weight. The tissue extracts were kept at $-20°$ until SMC assay was performed.

Somatomedin C radioimmunoassay

SMC was estimated 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. For standards and iodination a full amino acid sequence analog (AMGEN Biologicals, Thousand Oaks, Calif, USA) was purchased from Amersham (Buckinghamshire, UK).

All constituents were made up in 40 mmol/l phosphate buffer, pH 8.0, with 0.2% bovine serum albumin (Sigma, St Louis, Mo, USA) and sodium methiolate 0.6 mmol/l. Separation was achieved using (6:1) 20% polyethylene glycol 6000 with 0.5% Tween 20 (both from Merck, Darmstadt, FRG). Free and antibody-bound activities were counted.

SMC immunoactivity was measured in diluted rat serum (1:400) after previous extraction in acetic acid-methanol [14].

RNA, DNA and protein determination

RNA and DNA were separated by a Schmidt-Tannhäuser procedure performed according to Munro and Fleck [15]. DNA was determined with diphenylamine [16].

The protein concentration of the kidney homogenate was determined according to Lowry et al. [17] with bovine albumin as standard.

Statistical analysis

Statistics were performed using unpaired Student's t-test.

Results

All diabetic animals had blood glucose exceeding 15 mmol/l at day 1, and a mean level of 19 ± 1.0 mmol/l (SEM) which was maintained in the untreated group, while it was reduced to 6.2 ± 1.0 mmol/l in insulin treated animals. In untreated diabetic rats glucosuria ranged from 14-111 mmol/l. None of the animals had ketonuria exceeding 0.5 mmol/l.

Diabetic animals showed a small initial decrease in body weight, but at end of the study it was not significant. There was no change in body weight in uninephrectomised animals.

Significant differences between untreated and insulin treated diabetic groups of rats as well as between uninephrectomised and sham operated rats are shown in Figure 1.

As seen in Figure 1 A (left) kidney weight increased from 816 ± 21 mg (SEM) in control rats to 940 ± 42 mg at day 4 in untreated diabetic rats ($p < 0.020$), while insulin treated diabetic rats had unchanged kidney weight at 825 ± 15 mg after 4 days. Figure 1 A (right) illustrates the kidney growth following unilateral nephrectomy increasing form 840 ± 20 mg wet weight (SEM) to 1050 ± 60 mg at day 4 ($p = 0.010$).

The kidney content of protein, DNA and RNA was estimated. At day 4, there was an 11% increase of protein in untreated diabetic animals ($p = 0.009$) (Fig. 1 B (left)) and a 25% increase in uninephrectomised rats ($p = 0.003$) (Fig. 1 B (right)), which parallelled the increase pattern in wet kidney weight.

Figure 1 C shows an increase in RNA of 10% ($p = 0.05$) in non-insulin treated diabetic rats (left) and 35% ($p = 0.003$) in nephrectomised rats (right).

Untreated diabetic animals had a slight (but insignificant) increase in DNA during the observation period, while an increase of 17% was found in uninephrectomised animals ($p = 0.042$). Figure 1 D (left) shows an 8% increase in RNA/DNA ratio in untreated diabetic animals ($p = 0.048$) and an insignificant increase during insulin treatment. In Figure 1 D (right) an increase of 14% is shown in uninephrectomised rats ($p = 0.030$). Finally, protein/DNA ratio increased by 9% and 7%, respectively ($p = 0.030$ and $p = 0.041$).

Assuming that DNA content is an expression of the number of cells and the protein/DNA ratio an expression of the average cell size, it can be estimated that diabetic kidney growth was mainly due to cellular hypertrophy, while postnephrectomy kidney growth was due to roughly 60% hyperplasia and 40% hypertrophy.

Figure 1 E (left) illustrates the kidney content of SMC in diabetic rats. In reached maximum in untreated diabetic rats after 48 h and increased by 77% (1961 ± 173 ng/g (SEM) to 3469 ± 312 ng/g) ($p = 0.001$). The following two days the SMC content returned to the same level as in the non-diabetic control rats. When insulin treatment was started (one day

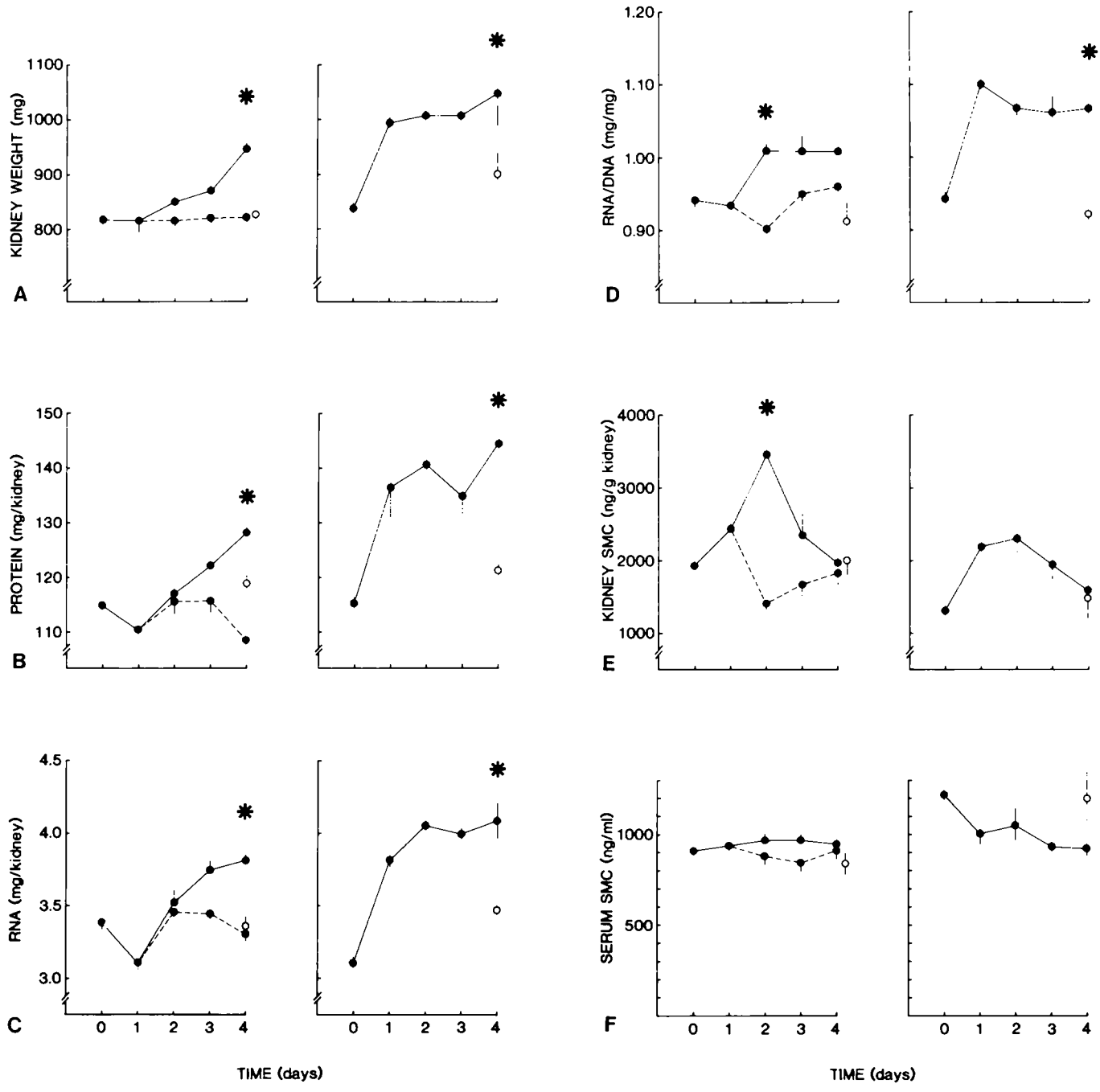

Fig. 1A-F. Changes over 4 days in streptozotocin diabetic rats (left) and unilaterally nephrectomised rats (right) in **A** kidney weight; **B** kidney protein; **C** kidney RNA; **D** kidney RNA/DNA ratio; **E** kidney somatomedin C (SMC); and **F** serum SMC. Each point represents 5 different animals ± SEM, except for day 0 in serum SMC, which includes all 45 rats in the diabetic study and all 30 rats in the uninephrectomy study. Untreated diabetic rats (●—●); insulin treated diabetic rats (●----●); non-diabetic control rats (○). Nephrectomised rats (●—●); sham operated rats (○).

* $p < 0.05$, between treated and untreated diabetic groups and between nephrectomised and sham operated rats

after streptozotocin injection) serum SMC content fell after the slight elevation at day 1 and settled around the concentration in control animals.

As shown in Figure 1 E (right) kidney SMC in the remaining kidney after uninephrectomy was maximal after 24 h, with an increase of 58% (from 1340 ± 203 ng/g (SEM) to 2122 ± 214 ng/g ($p = 0.025$). At day 4 the level was 1575 ± 229 ng/g compared to sham operated animals with a tissue content of 1527 ± 296 ng/g.

These results, however, do not exclude the theoretical possibility that sham operated rats might have exhibited a rise in the contralateral kidney SMC content after 24 h. We therefore performed an additional short-term study comparing 5 nephrectomised to 5 sham operated rats and found a kidney weight increase in the

former (796 ± 27 vs 700 ± 26 mg (SEM, $p = 0.05$) and no 24-h increase in the latter (712 ± 13, which was lower than 796 ± 27 mg, $p = 0.02$). Kidney SMC rose by 22% (from 1271 ± 61 to 1550 ± 70 ng/g, $p = 0.005$) while no 24 h increase was noted in sham operated rats (1148 ± 40 ng/g, which was lower than 1550 ± 70 ng/g, $p = 0.001$).

Figure 1 F shows serum SMC levels. In diabetic animals (insulin-treated as well as untreated) no significant changes were found (left). In uninephrectomised rats serum SMC concentrations fell from 1221 ± 34 ng/ml (SEM) at day 0 to 1000 ± 66 ng/ml at day 1 ($p = 0.02$) and remained at this lower level throughout the observation period (Fig. 1 F (right). Sham operated rats had a serum concentration of 1200 ± 177 ng/ml at day 4.

Discussion

Our study confirms previous observations that diabetes of recent onset as well as unilateral nephrectomy are followed by rapid kidney growth, involving both hypertrophy and hyperplasia [18, 19]. SMC is one of several growth factors having these effects, which would qualify it for being the "renotrophic factor". Severe experimental diabetes with general wasting, i.e. when blood glucose levels exceed 25 mmol/l, makes comparisons with control rats difficult and inhibits kidney growth [18, 20]; therefore, a moderate streptozotocin diabetes was induced in the present study.

It has been demonstrated that onset of diabetes is followed by decreased *serum levels* of SMC within one week, probably mainly due to impaired hepatic production, and that these changes are corrected by insulin treatment [21, 22]. In the present study no significant decrease was found in the untreated diabetic rats, possibly because of the short observation period and the comparatively mild degree of metabolic derangement.

Traditionally SMC was considered to originate in the liver, promoting growth at distant sites [10, 11]; but recent studies [12, 13] show that the somatomedins may act through paracrine or autocrine mechanisms, having major biological actions at or near peripheral sites of origin.

In a recent report, which addresses the potential role of local production of SMC in postnephrectomy renal growth in rats, tissue levels of SMC were increased after 5 days but no significant change was found after 24 h [23]. Serum SMC levels were unchanged.

We observed increased kidney content of SMC during initial kidney growth in diabetic as well as uninephrectomised rats. In the latter a prompt increase occurred, maximal after 24 h, in good accordance with the pronounced increase in kidney wet weight during the first 24 h following nephrectomy. In diabetic rats the SMC content was maximal after 48 h in accord with the somewhat slower increase in kidney weight; further, the increase in kidney size and SMC content was prevented by insulin treatment. This excluded also the possibility that the kidney SMC increase could be caused by streptozotocin.

It has recently been demonstrated that exogenous SMC, given to Snell dwarf mice, increases kidney weight [24]. However, in the present study kidney growth was not induced by increased liver production of SMC, as no elevation of serum SMC was observed. Still, the rise might, theoretically, be in part due to the greater blood volume of the hypertrophying kidney. In the study referred to above Stiles et al. [23] applied a correction factor using the haemoglobin concentration in the acetic acid extract and concluded that the contribution from plasma was insignificant. In our hands the concentration of haemoglobin in extracts was too low to allow for any meaningful correction. However, it can be calculated that the contents of the simultaneously expanding intravascular space can maximally account for about 2% of the 58% and 77% increase in tissue SMC found in the present experiments.

Possible explanations for the SMC increase include a passive capture of SMC secondary to the increase in renal plasma flow (RPF) and glomerular filtration rate (GFR) occurring after uninephrectomy as well as induction of malregulated diabetes, in keeping with the fact that the kidney is a major site for removal of plasma SMC [25]. However, kidney SMC increase patterns (Fig. 1 E) do not follow the known alterations in RPF and GFR in the two experimental situations [26–29].

Secondly, it could be that the kidney SMC is taken up from plasma after induction of an increased number of specific SMC receptors in kidney. This theory could be supported through binding studies in vitro. However, the rather stable serum SMC levels in the present experiments do not support this possibility.

Finally, and more intriguing in our view, would be an induction of augmented local formation of kidney SMC exerting paracrine effects; this could possibly also be substantiated in vitro.

It is well documented that glomeruli grow very quickly during the first four days of experimental diabetes, faster than the rest of the kidney [30]; this is absent in compensatory growth. In a study of ^{3}H-thymidine incorporation in kidneys of diabetic and uninephrectomised rats it was found that tubular growth was due to hyperplasia as well as hypertrophy while initial glomerular enlargement was due to hypertrophy only [31]. Noteworthy is that the rate of ^{3}H-thymidine incorporation was maximal 2 days after induction of diabetes or uninephrectomy, with a decline at day 4 and 6, similar to changes in the kidney SMC concentrations in the present study.

The rapid accumulation of potent mitogenic SMC in the present work and the studies of thymidine incorporation referred to above support a causal relation-

ship between kidney SMC increase and initial kidney growth. In other words, SMC may be the "renotrophic factor".

Compensatory kidney growth after uninephrectomy is relevant from a (patho)physiological point of view; but it is difficult to see any evident advantages in the similar abrupt increase in function and size after onset of imperfectly controlled diabetic metabolism. It has been suggested to be harmful; and possibly related to the development of late diabetic nephropathy [32-35].

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