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Obstructive nephropathy in the pig

Possible roles for Insulin-like growth factor I

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Summary. Kidney growth was investigated in 30-kg pigs after 72 h of unilateral ureteral obstruction. The data were compared to control kidneys from normal non-operated pigs at same weight. Kidney wet weight was determined. Cortex and medulla were separated, and from both regions RNA, DNA, protein and kidney tissue insulin-like growth factor I was determined. Unilateral obstruction caused a doubling of the wet hydronephrotic kidney weight and an ipsilateral 76% increase in total kidney protein content. RNA increased by 45% in the cortex and 76% in the medulla. Kidney protein in the contralateral cortex increased by 23% and RNA by 42%. In the hydronephrotic kidney DNA was reduced by 13% in the cortex and by 21% in the medulla. Contralaterally, DNA was the same as in the controls. Mean kidney insulin-like growth factor I increased sevenfold in the ipsilateral medulla but in the cortex it was the same as in the controls. Serum insulin-like growth factor I concentration was 1.7 $\pm$ 1.1 µg/l in the hydronephrotic animals and 1.2 $\pm$ 0.8 µg/l in controls. At this stage of obstruction, our data demonstrate (1) hydronephrotic growth that is most probably hyperplastic in the medulla, associated with an increase in medullary insulin-like growth factor I, (2) hyperplastic growth in the cortex, and (3) contralateral kidney growth that is mainly hypertrophic after 72 h of contralateral ureteral obstruction.

Key words: Insulin-like growth factor I – Obstructive nephropathy – Pigs – Kidney growth

Unilateral renal disease or unilateral nephrectomy results in profound changes in function and structure of the contralateral kidney. The events taking place are referred to as compensatory renal growth (CRG). Most studies on this phenomenon have been done in experimental models following unilateral nephrectomy. After unilateral total ureteral obstruction the contralateral kidney tissue undergoes the same types of structural and functional changes as after unilateral nephrectomy, but the time course is more prolonged and more highly age- and species-dependent than after nephrectomy [14].

In the hydronephrotic kidney however, the initial response to ureteral occlusion is an ipsilateral change in renal hemodynamics [10, 18, 26]. These changes have been ascribed to the participation of a number of vasoactive compounds within the hydronephrotic kidney. Concomitantly there is an increase in the mitotic index, mainly of the interstitial cells; this phenomenon is referred to as mesenchymal growth [19, 21, 24]. Fibroblasts cultured from the medulla of the obstructed rabbit kidney show an increased growth response [7, 26]. In contrast, the contralateral kidney enlarges as a result of hyperplasia and hypertrophy of the renal parenchyma, with the onset of the growth process delayed compared with the obstructed kidney. Therefore, it is obvious that some kind of growth stimulus is generated, acting on both the hydronephrotic kidney and the contralateral kidney, although the growth mode differ between the two sides. Apparently CRG does not begin until ipsilateral kidney function has been markedly impaired [22, 24]. Many studies have been performed in attempts to clarify what kind of stimulus is responsible for the initiation of kidney growth. Insulin-like growth factor I (IGF-I) is a polypeptide with potent mitogenic activity both in vitro [4, 31] and in vivo [11, 27, 28]. Recently, we have shown a possible connection between contralateral kidney growth and renal IGF-I (R-IGF-I) content in a rat model with unilateral hydronephrosis [16].

The aims of this study in pigs (porcine and human kidneys are similar) were to investigate: (1) whether R-IGF-I can be identified in the polypapillary pig kidney, (2) whether R-IGF-I has any potential relation to the initial kidney growth after unilateral ureteral obstruction, and (3) the biochemical nature of this growth.

Methods

Eight female pigs of the Danish land breed and with a mean body weight (b.w.) of kg were used. General anesthesia with halothane was induced with ketamine 50 mg/kg b.w. and midazolam 0.5 mg/kg

Table 1. Wet weight of kidneys at sacrifice and estimated total protein content and content of protein, RNA and DNA in cortex and medulla for the three kidneys (mean ± SE)

		IL	CL	C
Weight (g)		150.0 ± 15.3*	76.2 ± 6.6	62.5 ± 4.3
Protein (mg/g)	Cortex	81.1 ± 1.4*	113.2 ± 2.5	109.0 ± 2.3
	Medulla	72.8 ± 2.4*	94.3 ± 5.0	96.3 ± 2.8
RNA (mg/g)	Cortex	4.11 ± 0.10*	3.31 ± 0.13*	2.82 ± 0.08
	Medulla	4.06 ± 2.40*	2.87 ± 0.17	2.62 ± 0.12
DNA (mg/g)	Cortex	3.21 ± 0.10*	3.57 ± 0.19	3.68 ± 0.15
	Medulla	3.23 ± 0.21*	4.12 ± 0.25	4.09 ± 0.27
Total protein (g)		11.5 ± 1.2*	7.9 ± 0.6	6.4 ± 0.4

IL, Ipsilateral; CL, contralateral; C, control
*$P<0.05$

b.w., and the pigs were operated on using a sterile technique. Prior to operation the pigs were fed a standard diet. Food was withheld for 12 h before operation, but free access to water was maintained. A midline incision was made, exposing the kidneys extraperitoneally. In four pigs the left ureter was identified and a ligature tied around it 1 in. distal to the ureteral-pelvic junction creating a total unilateral ureter obstruction. In all animals, the wounds were closed in three layers. From another four pigs the four kidneys used for control studies were removed. At 72 h after unilateral ureter obstruction the remaining four pigs were anesthesized and both kidneys taken out.

All kidneys were weighed and divided into two identical parts. The medulla was separated from the cortex and the tissue kept at −20°C for R-IGF-I analysis.

IGF-I extraction from kidney

IGF-I extraction was performed as described by D'Ercole [8]. The frozen kidney was homogenized in 1 mol/l acetic acid (5 ml/g tissue) over an ice bath. With this procedure the pH range is 3.6–4.2, resulting in maximal liberation of IGF-I from the tissue. The extract was incubated on ice for 2 h and centrifuged at 4000 rpm for 15 min, after which the supernatant was decanted. The pellet was reextracted once, and the supernatants pooled and then 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. The tissue extracts were kept at −20°C until IGF-I assay.

IGF-I radioimmunoassay

IGF-I was estimated using IGF-I antibody UB 286 (raised by L. E. Underwood and J. J. van Wyk, Pediatric 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 analogue was used (AMGEN Biologicals, Thousand Oaks, Calif., USA; Amersham, Amersham Bucks., 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.

IGF-I immunoactivity was measured in serum after previous extraction in acetic acid-methanol [28].

RNA, DNA, and protein determination

RNA and DNA were separated by a Schmidt-Tannhäuser procedure performed according to Munro and Fleck [20]. DNA was determined with dephenylamine [3].

The protein concentration in the kidney homogenate was determined according to Lowry et al. [15] with bovine albumin as standard.

Total protein content, TPC, of the kidney was estimated by

$$TPC = \text{Kidney weight} \times [PC(M) + PC(C)/2]$$

where PC(M) is the protein concentration in medulla and PC(C) is the protein concentration in cortex.

Statistical analysis

All data are presented as means ± SEM. Comparisons were made by means of the Mann-Whitney test for unpaired data. *P*-values smaller than 0.05 were considered significant.

Results

All kidneys were macroscopically normal at the initial operation. After 72 h of obstruction the ipsilateral kidney weight was 150.0 g ± 15.3 g, as against 62.5 g ± 4.3 g in normal kidneys ($P<0.001$) (Table 1). The contralateral kidneys weighed 76.2 g ± 6.6 g. No change in the body weight of any animal was observed during the study.

The mean ipsilateral IGF-I concentration was 3.4 ± 1.6 ng/g kidney wet weight in the cortex and 17.3 ± 5.5 ng/g in the medulla (Fig. 1). Contralaterally, R-IGF-I was 3.8 ± 1.5 ng/g in the cortex and 3.3 ± 0.9 ng/g in the medulla. In control kidneys the corresponding values were 2.4 ± 1.0 ng/g in the cortex and 1.9 ± 0.3 ng/g in the medulla. The ipsilateral IGF-I content in the medulla was significantly higher than in control kidneys ($P<0.01$). Serum IGF-I levels were 1.7 ± 1.1 μg/l in the hydronephrotic animals and 1.2 ± 0.8 μg/l in the control animals.

Tissue relative protein content (Table 1) was significantly lower in both cortex and medulla than in controls,

Table 2. Ratios between RNA/DNA and protein/DNA in cortex and medulla for the three kidneys (mean ± SE)

		IL	CL	C
RNA/DNA	Cortex	1.28 ± 0.05*	0.95 ± 0.06	0.80 ± 0.04
	Medulla	1.25 ± 0.06*	0.70 ± 0.04	0.65 ± 0.05
Protein/DNA	Cortex	25.28 ± 0.38*	32.68 ± 1.54	29.70 ± 1.01
	Medulla	22.95 ± 1.64	22.98 ± 0.97	23.75 ± 1.31

*$P<0.05$

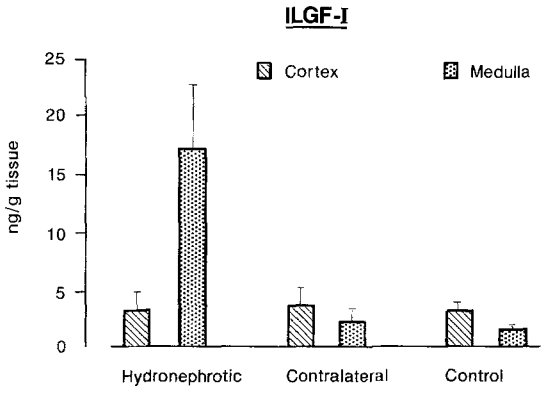

Fig. 1. Tissue concentrations (means ± SEM) of insulin-like growth factor I in cortex and medulla of hydronephrotic, contralateral and control kidneys

whereas no difference was found between contralateral and control kidneys. However, the results displayed in Table 1 show that the total protein content in both the hydronephrotic kidney and the contralateral kidney was significantly higher.

RNA analysis (Table 1) showed a cortical content of 2.82 ± 0.08, as opposed to 4.11 ± 0.10 in the controls ($P<0.05$). RNA in the medulla was 2.62 ± 0.12 in hydronephrotic kidneys and 4.06 ± 2.40 in control ($P<0.02$). A significant difference in DNA content was found in the cortex 3.68 ± 0.15 versus 3.21 ± 0.10 ($P<0.05$)) and in the medulla it was 4.09 ± 0.27, as against 3.23 ± 0.21 ($P<0.05$). No change in RNA and DNA content was found in contralateral kidneys compared with controls.

From the above data the ratios between RNA/DNA and Protein/DNA were calculated (Table 2). In the hydronephrotic kidneys a significant difference was seen in the RNA/DNA ratio in both the cortex (60%) and medulla (92%) compared with the controls. Likewise, a significant difference (15%) from controls was found in the cortical protein/DNA ratio.

Discussion

From the present study it is apparent that unilateral total ureteral obstruction for 3 days in the pig results in a rapid increase in ipsilateral kidney weight. This finding concurs with results reported in rat [9] and mouse studies [23, 25]. Part of this weight increase is due to increased water content in the hydronephrotic kidney, and wet kidney weight is therefore not a reliable parameter of the degree of kidney growth. This is clearly demonstrated in this study, in which we found a lower relative content of protein in the hydronephrotic kidney compared with control kidneys. However, a net increase in total kidney protein was seen, which demonstrates that the weight increase is not only water dependent. The RNA and DNA analysis yielded further evidence that the hydronephrotic kidney underwent true growth. The RNA content of the hydronephrotic kidney was 46% higher in the cortex and 55% higher in the medulla after 72 h of obstruction than in controls. This reflects an activated mitotic turnover accompanied by a hypertrophic response, as evidenced by RNA/DNA ratios of 1.28 in cortex and 1.25 in medulla as against RNA/DNA ratios of 0.80 in cortex and 0.65 in medulla of kidneys from controls. The protein/DNA ratio yields information on the mean cell size. In the hydronephrotic kidneys the protein/DNA ratio was slightly reduced in the cortex but unchanged in the medulla compared with controls. This suggests that the growth response seen in the hydronephrotic kidney is mainly hyperplastic at this stage. The lower protein content per gram of kidney tissue in the hydronephrotic kidney concurs with the time-dependent changes observed in previous studies, where renal protein increased in the obstructed mouse kidney 24 h after ureter ligation and then began to decline [24].

We found a significantly higher medullary content of IGF-I in the hydronephrotic kidneys. Recent studies have shown that IGF-I is synthesized in many tissues [8]. The action of IGF-I is controversial. Some investigators suggest that IGF-I acts locally where it is synthesized, as an autocoid, and that plasma IGF-I does not play a physiological role [8, 29], whereas other investigators suggest that there is a hormonal action of IGF-I [12]. This study demonstrates a local medullary increase in the production of IGF-I of the hydronephrotic kidney, which might be involved in the hyperplastic growth. However, in the present study we were not able to characterize or quantitate the type of cells involved in the hydronephrotic growth response, but the hyperplastic response we have demonstrated biochemically is in accordance with the histological changes previously been found in the hydronephrotic pig kidney [17] and rabbit kidney [21], which may reflect an increase in the number of fibroblasts and monocytes.

The cortical growth response was not associated with an increase in cortical IGF-I concentration. However, previous studies have shown increased growth of cultured fibroblasts from the cortex and outer medulla of the obstructed rabbit kidney compared with contralateral and control kidneys [21, 26, 32]. Thus, total 72 h obstruction could cause a stimulus for increased production of IGF-I in the renal medulla that in terms triggers a regional inflammatory response resulting in stimulation of the interstitial cell proliferation and mononuclear cell invasion. This could suggest that the growth is a local response to injury, as proposed previously [32].

On the non obstructed side there was no significant difference in kidney weight compared to controls. However, it appears from clinical [6, 13] and experimental [1, 14] studies that CRG depends on both age and the severity of the contralateral obstruction. Therefore, CRG may begin in response to obstructive nephropathy rather than to loss of function [22, 24], and previous studies have shown a range from 24 h [2] to 7 days [1] before growth is initiated.

In non obstructed kidneys protein content per gram kidney tissue was unchanged compared to controls. The RNA content in the cortex was significantly higher, whereas RNA in the medulla and DNA in both regions was the same. The RNA/DNA ratio in the cortex was higher than in controls. From these data it is evident that growth is stimulated in accordance with results from previous studies [2, 23–25]. However, from the present data we are not able to conclude what kind of cells were proliferating, but at this point our results are consistent with the finding of Josephson et al. [14] and could reflect a primarily increase in cell size and to a lesser extent an increase in the number of cells at this stage of growth. Since IGF-I is a mitogenic substance it is not surprising that there is an unchanged kidney tissue IGF-I level at this time after contralateral obstruction. In a previous study in rats [16], we suggested that IGF-I may be involved initially, since CRG is preceded by a rise in the concentration of IGF-I. However, we found no evidence for any major hyperplastic response at this time of CRG in pigs.

Recent theories have proposed that the IGF-I content in the kidney could be caused by entrapment of circulating IGF-I. However, the serum IGF-I levels were extremely low, and since renal plasma flow is also reduced during obstruction we do not conceive of it as the reason for the observed level of tissue IGF-I. IGF-I taken up from plasma after induction of an increased number of IGF-I receptors in the obstructed kidney could increase tissue IGF-I. Previous investigations have shown that cultured fibroblast contain a high concentration of IGF-I binding proteins [5]. Since it is possible that part of the cell increase is caused by migration of fibroblasts to the hydronephrotic kidney, we are not able to exclude a contribution of these cells to the total amount of R-IGF-I.

In conclusion this study has shown that unilateral complete obstruction of the ureter for 72 h has a tremendous impact on ipsilateral kidney weight, which is caused by an increased water content as well as true tissue growth. This is evidenced by the changes in the content of protein, RNA and DNA. We also found that the hydronephrotic kidney medulla contained significantly more IGF-I than control kidneys. Our study therefore suggest a possible connection between R-IGF-I and the initial hyperplastic growth in the medulla and cortex of the hydronephrotic kidney which we have demonstrated biochemically. However, the role of IGF-I in contralateral compensatory kidney growth is unclear. No increase in kidney tissue IGF-I was found on this side, and at this stage CRG seems to be explained by cell hypertrophy rather than hyperplasia. Future studies will determine the definite role of IGF-I in both ipsilateral and contralateral growth.

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References

1. Ardison GW (1965) Compensatory renal hyperplasia in rats rendered hydronephrotic by unilateral ureteral ligation. Res Rev Bowman Gray Sch Med 5:61
2. Benitez L, Shaka JA (1964) Cell proliferation in experimental hydronephrosis and compensatory renal hyperplasia. Am J Pathol 44:961
3. Burton K (1956) A study of the conditions and mechanism of the dephenylamine reaction for a colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315
4. Clemmons DR, Underwood LE, van Wyk JJ (1981) Hormonal control of immunoreactive somatomedin production by cultured human fibroblasts. J Clin Invest 67:10
5. Conover CA, Liu F, Powell D, Rosenfeld RG, Hintz RL (1989) Insulin-like growth factor binding proteins from cultured human fibroblasts. Characterization and hormonal regulation. J Clin Invest 83:852
6. Davies P, Roylance J, Gordon IRS (1972) Hydronephrosis. Clin Radiol 23:312
7. Davis BB, Thomasson D, Zenser TV (1983) Renal disease profoundly alters cortical interstitial function. Kidney Int 23:458
8. D'Ercole AJ, Stiles AD, Underwood LE (1984) Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of actions. Proc Natl Acad Sci USA 81:935
9. Dicker SE, Shirley DG (1972) Compensatory hypertrophy of the contralateral kidney after unilateral ureteral ligation. J Physiol (Lond) 220:199
10. Frøkiær J, Jensen FT, Husted SE, Mortensen J, Djurhuus JC (1988) Renal blood flow and pelvic pressure after 4 weeks of total upper urinary tract obstruction in the pig. The effect of a TxA_2 synthetase inhibitor on active preglomerular vasoconstriction. Urol Res 16:167
11. Hammerman M (1989) The growth hormone-insulin-like growth factor axis in kidney. Am J Physiol 257:F503
12. Hirschberg R, Kopple JD (1989) Evidence that insulin-like growth factor I increases renal plasma flow and glomerular filtration rate in fasted rats. J Clin Invest 83:326
13. Hodson CJ, Craven JD (1966) The radiology of obstructive atrophy of the kidney. Clin Radiol 17:305
14. Josephson S, Robertson B, Claesson G, Wikstad I (1980) Experimental obstructive hydronephrosis in newborn rats: I. Surgical technique and longterm morphologic effects. Invest Urol 17:478
15. Lowry OH, Osebrough NJ, Fara AL, Randall RL (1951) Porcine measurements with the Folin phenol reagents. J Biol Chem 193:165

16. Marshall SM, Flyvbjerg A, Frøkiær J, Ørskov H (1991) The role of insulin-like growth factor I in renal growth following unilateral ureteral obstruction. Nephron 58:219
17. Møller JCM (1986) Dimensional changes of proximal tubules and cortical capillaries in chronic obstructive renal disease. A light microscopic morphometric analysis. Virchows Arch [Pathol Anat] 410:153
18. Moody TE, Vaughan ED Jr, Gillenwater JY (1975) Relationship between renal blood flow and ureteral pressure during 18 hours of total unilateral occlusion: implications for changing sites of renal resistance. Invest Urol 13:246
19. Mullin EM, Bonar RA, Paulson DF (1975) Effect of transient hydronephrosis on subsequent compensatory renal growth. Invest Urol 13:121
20. Munro HN, Fleck A (1966) The determination of nucleic acids. Methods Biochem Anal 14:113
21. Nagle RB, Johnson ME, Jervis HR (1976) Proliferation of renal interstitial cells following injury induced by ureteral obstruction. Lab Invest 35:18
22. Obertorp H, Malt RA (1977) Lost mass and excreation as stimuli to parabiotic compensatory renal hypertrophy. Am J Physiol 232:F405
23. Paulson DF, Fraley EE (1970) Chemical evidence for early but unsustained growth in the obstructed mouse kidney. Am J Physiol 219:872
24. Paulson DF, Fraley EE (1973) Compensatory renal growth after unilateral ureteral obstruction. Kidney Int 4:22
25. Paulson DF, Fraley EE (1971) Sequential changes in bulk renal protein and polyribosomes in the acutely obstructed mouse kidney. J Urol 103:257
26. Preuss HG (1983) Compensatory renal growth symposium: an introduction. Kidney Int 23:571
27. Schoenle E, Zapf J, Humbel RE, Froesch ER (1982) Insulin-like growth factor I stimulates growth in hypophysectomized rats. Nature 296:252
28. Thorlacius-Ussing O, Flyvbjerg A, Esmann J (1987) Evidence that selenium induces growth retardation through reduced growth hormone and SMC production. Endocrinology 120:659
29. Underwood LA, D'Ercole AJ, Clemmons D, van Wyk JJ (1986) Paracrine functions of somatomedins. Clin Endocrinol Metab 15:59
30. Vaughan ED Jr, Sorensen EJ, Gillenwater JY (1970) The renal hemodynamic response to chronic unilateral complete ureteral occlusion. Invest Urol 8:78
31. Zapf J, Froesch ER, Humbel RE (1981) The IGF of human serum: chemical and biological characterization and aspects of their possible physiological role. Curr Top Cell Regul 19:257
32. Zelman SJ, Zenser TV, Davis BB (1983) Renal growth in response to unilateral ureteral obstruction. Kidney Int 23:594

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