UNILATERAL URETERIC LIGATION IN DIABETIC RATS: A PATHOLOGIC AND MORPHOMETRIC STUDY

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Summary

We studied the combined effect of unilateral ureteral ligation on the morphology and pathology of the contralateral kidney in normal and streptozotocin induced diabetic rats. In the diabetic group that underwent ligation of the right ureter the weight and volume of the left kidney was far greater, the tissue specific gravity far lower, while the percentage of affected glomeruli was significantly greater compared with control rats and those undergoing only ureteric ligation.

Introduction

It is well known that unilateral ureteral obstruction, ligation, or nephrectomy induces hypertrophy of the contralateral kidney. It is also known that diabetes mellitus causes bilateral renal hypertrophy [1,2].

To date however, no one has studied the combined effect of unilateral ligation and diabetes on the fate of the contralateral kidney. Therefore we studied this effect and we report here our findings on the weight, volume, tissue specific gravity (t.SG) and the pathological changes in the contralateral kidney.

Material and methods

In this study we used 31 female wistar rats weighing between 180 and 210 grams. The rats were divided into four groups:

Group 1 Control (C): Intact normal rats (n = 8); Group 2 Diabetic (D): Streptozotocin induced diabetic rats (n = 8); Group 3 Ligated (L): Normal rats whose right ureter was ligated surgically with 2.0 silk suture at the site of the ureterovesical junction; Group 4 Ligated plus Diabetic (DL): The right ureter of the rats ligated as in group 3 (n = 7). The experimental diabetes mellitus was evoked two days later by bolus IV streptozotocin injection via the dorsal tail
vein, the dose being 45mg/kg BW and, the vehicle being buffered citric acid in 0.9 per cent saline PH : 4.6. All rats were fed (Purina chow rat, pellets) and had free access to water. The experiment lasted 35 days from the ureteral ligation. The criterion for inclusion in the diabetic group was a blood glucose above 250mg/dl estimated at day seven and day 35 of the experiment. Diabetic rats were not treated with insulin or any other drugs. On the 35th day of the experiment the rats were anaesthetised with ether, a blood sample was drawn from the tail, and, the left kidney was surgically removed. The excised kidneys were trimmed free of fat, measurements (weight, volume) were obtained and the tissue specific gravity calculated. Following this the kidneys were processed for pathological and microscopic examination for H/H, PAS, and van Gieson orcein stains. In the microscopic examination increase of mesangial tissue was measured from a study of 20 randomly selected glomeruli. Statistical evaluation was done by Student’s ‘t’ test.

Results

The morphological parameters of the kidneys of the studied rats of each group are shown in Table I. The rats in the group L, D and DL have larger kidneys as measured by weight and volume than the controls. In more detail the DL group had kidneys nearly twice the weight of the controls and larger than rats in groups D and L (p<0.005 and p<0.001).

**TABLE I. Morphometric measurements of left kidney in normal and diabetic rats with or without ligation of the right ureter**

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Weight mg</th>
<th>Volume ml</th>
<th>t.SG mg/cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>8</td>
<td>689.12 ± 23.2</td>
<td>0.70 ± 0.02</td>
<td>980.95 ± 10.33</td>
</tr>
<tr>
<td>L</td>
<td>8</td>
<td>1096.50 ± 31.81</td>
<td>1.18 ± 0.04</td>
<td>927.41 ± 4.47</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>1028.75 ± 35.48</td>
<td>0.93 ± 0.03</td>
<td>1100.30 ± 11.62</td>
</tr>
<tr>
<td>DL</td>
<td>7</td>
<td>1236.29 ± 42.28</td>
<td>1.37 ± 0.04</td>
<td>900.51 ± 12.7</td>
</tr>
</tbody>
</table>

* X ± SD

The volume of groups L, D and DL left kidneys were greater than controls. DL kidneys were largest followed by L and D (p<0.001).

The tissue specific gravity of D group kidneys was greater than controls while L and DL group kidneys were smaller than control kidneys (lowest t.SG kidneys in DL group. All differences significant (p<0.005 to p<0.001) (Figure 1).

**Histological results**

In all groups increase in mesangial tissue and thickening of arteriolar walls was observed. Increase in mesangial tissue was more extensive in the DL group and least in group L (Figure 2).
Figure 1. Histograms showing variation in weight, tissue specific gravity and volume in the four groups of experimental animals.

Figure 2. Glomerulus from left kidney of group DL rats showing increased mesangial tissue. PAS x 400 – reduced for publication.
Discussion

Both diabetes mellitus and unilateral ureteral ligation are known to cause renal hypertrophy, bilaterally in the case of diabetes and of the contralateral kidney in the case of ureteral ligation [3]. The combined effect of both these defects on the contralateral kidney to the ligation have not yet been studied. The diabetic changes observed are seen at blood sugar values up to 450mg per cent [4]. The criterion for inclusion of the rats in the experimental groups was a blood sugar between 250 and 320mg per cent for the major part of the study period. In experimental diabetes mellitus the renal changes observed are most intense in the first month after induction of the disease [4]. The diabetic rats had renal weight increase of 32.8 per cent, the ligated rats of 59.1 per cent and the DL rats had renal weight increase of 79.4 per cent in comparison with controls. The diabetic rats had renal volume increase of 49.2 per cent, the ligated 68.5 per cent and the DL, 95.7 per cent again in comparison with controls.

It seems therefore, that the combination of diabetes and ligation have roughly additive effect on the weight and volume changes in the kidney. The mechanism by which this increase in weight and volume occurs in both these conditions is not clear. The hyperglycaemia has been blamed for the hypertrophy of DM and in unilateral ligation the renal secretion of a renotropic factor [5] by the obstructed kidney has been implicated and other factors such as growth hormone, prostaglandin, catecholamines and haemodynamic parameters [6] have also been suggested as being involved in the hypertrophy resulting from diabetes mellitus.

Figure 3. Proximal convoluted tubule of group DL rat showing hypertrophy and hyperplasia of cells. Some Armani Ebstein cells are shown. H/H x 400 – reduced for publication
Group D kidneys had a 12.7 per cent increase in tissue specific gravity while groups L and DL had 5.5 per cent and 8.2 per cent decrease in comparison with the control groups. Such results have not been reported in earlier studies. These results would support the hypothesis that kidneys in groups L and DL contained an increased perfusion of low s.g. substances such as fat or water. Histology excluded interstitial oedema. The mechanism would seem to be an increase in the filtrate present in tubular hypertrophy. The intra-tubular space is increased resulting in an increased content of filtrate and the results of the histological examination supports this view.

The greater change in tissue specific gravity in groups DL compared with group L may be accounted for by a) the greater degree of tubular hyperplasia observed in this group (Figure 3) and b) the occurrence of glucose in the tubules and thus the hyperplastic tubules contained for osmotic reasons a further increased load.

The increase in tissue specific gravity of the D group was attributed to an increase of solid material in the kidney. The increase in Armani-Ebstein cells in the DL as compared to the D group is not explained by variation in blood sugar values between these two groups as these were comparable, but the combination of ligation and diabetes mellitus causes an increase in the pathological changes as with diabetes, by mechanisms at present unknown.

References

5 Preuss A, Goldin H. Kidney Int 1983; 23: 635

Open Discussion

Di PAOLO (Chieti) According to the hypothesis of Viberti* and Brenner† I expect a decrease of matrix only in diabetic obstructed kidneys. You showed an increase of matrix in these kidneys. Have you any explanation for this?

HATZIGEORGIΟU I cannot give any explanation because I have not had the opportunity of electron microscopic examination.

CHAIRMAN You have carried out your experiments 35 days after ligation, but at this time, at least to my knowledge, the kidney is totally destroyed.

HATZIGEORGIΟU We ligate the right kidney and we study the left kidney. The right kidney is completely destroyed. There is no longer any function in the right kidney.

CHAIRMAN Why did you choose this model and not a nephrectomy model?
HATZIGEORGIOU We did not find in the literature any other type of protocol.

CHAIRMAN It has been shown that after two weeks of complete ureteral obstruction the kidney is no longer functioning and the tissue is fibrotic.

HATZIGEORGIOU Yes, after two weeks the right kidney is destroyed completely.