Age-dependent difference of kidney response to temporary ischaemia in the rat

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Summary

1. The response to 60 min ischaemia was studied in male uninephrectomized 30-, 60- and 90-day-old rats in regard to changes of renal cytochrome oxidase activity and the effect on systemic arterial blood pressure. Mortality due to renal failure, changes in plasma urea concentration and in kidney morphology were used as indicators of the renal response.

2. Mortality was lowest in 30-day-old animals (13%) and highest in 60-day-old animals (77%). Renal cytochrome oxidase activity increased by 44% during the same period. The mortality decreased to 34% between the ages of 60 and 90 days without change in renal content of cytochrome oxidase; survival time after renal ischaemia was prolonged from 2 to 4 days.

3. Fifty days after renal ischaemia plasma urea concentration correlated positively with kidney weight. Both variables were increased in rats exposed to ischaemia at the ages of 60 and 90 days. The kidneys of these rats exhibited lesions. No relation was found between the degree of renal damage and blood pressure.

4. It is concluded that (a) the kidney of immature rats is more resistant to temporary ischaemia due to immaturity of renal oxidative metabolism, (b) the ability to survive ischaemic renal damage in less-resistant mature rats increases with age and (c) the postischaemic impairment of renal function does not influence systemic arterial blood pressure.

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Introduction

In various mammals of different body size resistance of the kidney to temporary ischaemia increases with body weight (Oetliker, 1961). This relation, however, may not apply for growth-dependent changes in body weight during postnatal ontogeny, since developmental changes in renal oxidative metabolism might interfere. In the dog and rat oxygen consumption of the kidney tissue is lower in immature than adult animals (Čapek & Kleinzeller, 1961). Thus both changes in body weight and oxidative metabolism of kidney tissue may influence the kidney response to temporary ischaemia during postnatal ontogeny. This might be related to the body-weight-dependent increase of the hypertensive response to renal ischaemia in the rat, elicited by ligation of the aorta between the origin of renal arteries (Rojo-Ortega & Genest, 1968). The results of renal transplantsations might also be influenced by the age-dependent differences in oxygen demands of renal tissue as well as the homeostatic requirements of the organism. There are, however, no data dealing with this problem systematically. For this reason, we studied the age-dependent differences in the kidney response to temporary ischaemia, and the associated changes in arterial blood pressure in the rat, in relation to the renal oxidative metabolism.

Methods

We studied male Wistar-strain rats, fed on a standard diet containing 175 mmol of Na\(^+\) and 204
mmol of K+/kg and tap water ad libitum. At the ages of 30, 60 or 90 days one kidney was removed under ether anaesthesia and the pedicle of the remaining kidney was clamped with a bulldog clamp for 1 h in the experimental group. Preliminary experiments have shown that this procedure is sufficient to occlude the renal artery completely in all three age groups, as shown by the lack of penetration of intravenously injected Evans blue into the clamped kidney. Uninephrectomized rats with sham-operated remaining kidneys served as control animals.

The impairment of renal function was studied by determining plasma urea concentration in samples (0.1 ml) of blood plasma (Caraway & Fauger, 1956). Blood was obtained from the tail of the rat 1, 3, 6, 12 and 24 days after the operation and from the carotid artery at the end of the experiment (day 50).

Fifty days after the operation the animals were weighed and the mean blood pressure was measured under light ether anaesthesia by direct puncture of carotid artery, a Statham P23Db transducer and Gallileo recorder being used. The animals were then killed, the ischaemic kidney was weighed (to ± 2 mg) and representative samples were fixed in neutral formol for histological examination.

As an indicator of age-related differences in oxidative metabolism of kidney tissue, cytochrome oxidase (EC 1.9.3.1) activity was determined by an oxygen electrode (Yellow Springs, Ohio, U.S.A.) at pH 7.4 and 30°C (Smith & Camerino, 1963) in homogenates of kidneys, removed at the start of the experiments. One unit of cytochrome oxidase activity was expressed as the amount of enzyme utilizing 1 µg-atom of O per min.

For statistical evaluation, the Duncan’s test for contrasts, the Student's t-test and X2-test were used. The coefficients of linear correlations were calculated in the standard manner.

Results

Mortality and plasma urea

Mortality due to ischaemic renal damage differed significantly in all three age groups (P < 0.01), being lowest in the group exposed to the transitory ischaemia at an age of 30 days and highest in those exposed at an age of 60 days (Table 1). The animals died in severe uraemia, with a more than fivefold increase of plasma urea concentration. In the group exposed at the age of 90 days plasma urea concentration was higher than in both other groups (P < 0.001), perhaps because of the longer average time of survival after the renal ischaemia (P < 0.01).

In all the experimental animals which had survived the renal ischaemia a similar increase in plasma urea concentration was found on day 1 of the experiment (Fig. 1). In the groups exposed at ages 60 and 90 days, this increase continued up to the third day (P < 0.01) and was then followed until day 12 by a marked fall (P < 0.01) to values which remained above those in the control group, until the end of the experiment (P < 0.05). On the other hand in the group exposed at the age of 30 days a fall to values no different from those of the control rats appeared as early as between day 1

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Mortality %</th>
<th>Survival time* (days)</th>
<th>Plasma urea (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>13.2</td>
<td>7</td>
<td>1.69; 1.31, 2.18</td>
</tr>
<tr>
<td></td>
<td>(53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>76.8</td>
<td>43</td>
<td>2.26; 1.99, 2.58</td>
</tr>
<tr>
<td></td>
<td>(56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>35.4</td>
<td>23</td>
<td>4.43; 3.50, 5.56</td>
</tr>
<tr>
<td></td>
<td>(65)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean values with lower and upper 90% confidence limits.

Fig. 1. Time course of plasma urea concentration in rats which survived transient renal ischaemia elicited at different ages. Mean results ± SEM are shown for experimental (△, □, ○) and control (△, □, ○) rats at 30 (△, □), 60 (□, □) and 90 (○, ○) days of age, at which ages renal ischaemia was induced. For the numbers of animals see Table 2.
and day 6, the response to renal ischaemia being thus less pronounced than in older rats.

**Kidney weight and blood pressure**

In rats exposed to transient renal ischaemia at the ages of 60 and 90 days, the final kidney weights (after 50 days) increased above values found both in animals exposed at the age of 30 days as well as in all control groups \((P < 0.01)\) by approximately 20 and 40% respectively (Table 2). The higher kidney weights were associated with higher final plasma urea concentrations, as shown by positive correlation between both these variables \((r = 0.720, n = 100; P < 0.01)\). The mean blood pressure was slightly higher than control values in animals exposed at the age of 30 \((P < 0.05)\) and 60 \((P < 0.1, \text{n.s.})\) days. Such a difference was not found in animals exposed at the age of 90 days, since control values in this group were high.

**Cytochrome oxidase activity (Fig. 2)**

Cytochrome oxidase activity of kidney tissue per mg of protein changed with age, increasing between days 30 and 60 \((P < 0.01)\) and thereafter declining. The mean value found in 90-day-old rats was lower than in 60-day-old animals \((P < 0.01)\) but higher than in 30-day-old animals \((P < 0.01)\). The total cytochrome oxidase activity in kidneys, however, only increased between the age of 30 and 60 days, in parallel with the total amount of kidney protein \((P < 0.01)\). Between the ages of 60 and 90 days total cytochrome oxidase activity did not change, whereas total kidney protein continued to increase \((P < 0.01)\), thus causing a decline of cytochrome oxidase activity per mg of protein.

**Kidney morphology**

In kidneys subjected to ischaemia there were focal lesions of various degree located mainly in the juxtamedullary region of the renal cortex. The lesions involved renal tubules (calcified necrotic epithelia, atrophy and cystic dilatation accompanied by an increase of interstitial tissue) as well as glomeruli (thickening of Bowman's capsule, collapse of capillary loops) and were interspersed with hypertrophied tissue. Occasionally focal adhesions and hypercellularity of glomerular capillaries in both normal and damaged tissue were observed.

According to severity and extent the lesions were divided semiquantitatively into three groups: mild (focal tubular atrophy and occasional calcification

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### Table 2. Kidney weight, plasma urea and arterial mean blood pressure in rats surviving for 50 days after transient renal ischaemia elicited at different ages

<table>
<thead>
<tr>
<th>Age group (days)</th>
<th>n</th>
<th>Kidney wt. (mg/100 g body wt.)</th>
<th>Plasma urea (mmol/l)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 Experimental</td>
<td>46</td>
<td>508 ± 8.8</td>
<td>11.1 ± 0.49</td>
<td>130 ± 1.4</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>451 ± 21.2</td>
<td>9.8 ± 0.33</td>
<td>124 ± 2.3</td>
</tr>
<tr>
<td>60 Experimental</td>
<td>13</td>
<td>629 ± 38.6</td>
<td>17.7 ± 2.45</td>
<td>128 ± 3.4</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>442 ± 9.4</td>
<td>10.2 ± 0.35</td>
<td>123 ± 2.0</td>
</tr>
<tr>
<td>90 Experimental</td>
<td>42</td>
<td>599 ± 20.3</td>
<td>15.7 ± 1.16</td>
<td>129 ± 1.5</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>443 ± 22.3</td>
<td>10.3 ± 0.48</td>
<td>130 ± 2.3</td>
</tr>
</tbody>
</table>

\(n\) = Number of values in each group. Results are mean values ± SEM.
in less than 10% of cortical tissue: grade 1), moderate (the changes described above accompanied by dilatation of atrophic tubules, extending to less than 50% of renal cortex: grade 2), and severe (the changes in more than 50% of cortical tissue: grade 3). In both group 2 and group 3 the undamaged remnants of renal cortex exhibited marked hypertrophy and in the medulla numerous hyaline casts were present. Grade of changes was related to the age at which the kidney was exposed to the ischaemia, mild changes prevailing in the youngest, severe changes being present in both older groups (Fig. 3).

In rats exposed at 30 days of age the basal metabolic rate expressed in terms of body weight is higher by 30% (Brody, 1945); the mortality due to renal ischaemia, however, was lower than in the group exposed at 60 days by 83%. This difference could be due to the increase in aerobic metabolism of kidney tissue found in the rat between the age of 30 and 60 days (Caldwell & Solomon, 1975; Čapek, Drahota, Jelinek & Kuneš, 1976) and is also manifested by the increase of renal cytochrome oxidase activity found at this time.

The reaction of blood pressure to transient renal ischaemia was a slight and inconsistent elevation, which bore no clear relation to the age at which the kidney was exposed to ischaemia, or to ischaemic damage (as evaluated by plasma urea concentrations, kidney weight and histological data). Higher kidney weight was associated with higher plasma urea concentrations, which indicated a direct relationship between kidney weight and ischaemic damage. However, we found no correlation with blood pressure. We conclude that age-dependent changes in kidney sensitivity to ischaemic injury are not related to the age-dependent differences in the hypertensive response to ligature of the aorta between the renal arteries (Rojo-Ortega & Genest, 1968).

**References**


**Discussion**

There is evidence that the resistance of the kidney to transient ischaemia in different mammals increases with body weight due to differences in basal metabolic rate (Oetliker, 1961). Our results indicate that this relation is valid during postnatal development, but only from a certain degree of maturity onwards. Thus in the rats exposed to transitory renal ischaemia at the age 60 days, the mortality was higher and the time interval between ischaemic kidney injury and death was shorter than in rats exposed at the age of 90 days which were 30 days older and 50% heavier. During this age period the basal metabolic rate, expressed in terms of body weight, fell by approximately 30%.

*Fig. 3. Incidence of kidney lesions in animals exposed to renal ischaemia at the age of 30, 60 or 90 days. The incidence is expressed as a percentage of all animals in individual groups: 0 = no lesion; 1, 2, 3 = grades of kidney lesion (mild, moderate and severe). The number of animals in each group is given in parentheses. n.s., Not significant.*