Aggravation of hypertension in spontaneously hypertensive rats by Heymann nephritis

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Summary

1. To explore the effect of nephritis on development of genetic hypertension we immunized 10-week-old spontaneously hypertensive rats with purified rat kidney brush-border antigen. This induces Heymann nephritis (autologous immune complex nephritis), which does not elevate blood pressure in normal rats.

2. Nephritis developed in 11 of the 12 immunized animals, and systolic blood pressure rose to a significantly higher level than in the non-immunized spontaneously hypertensive rats within 4 weeks. Blood pressure remained higher in the immunized rats at 17 weeks, heart weights were greater, but creatinine clearance remained unchanged.

3. At 6 weeks, urinary sodium excretion was greater in the immunized spontaneously hypertensive rats, whereas at 17 weeks, sodium excretion was decreased in these animals along with reduced serum protein concentration, packed cell volume and plasma renin activity, as compared with that of the controls.

4. Development of hypertension in nephritic rats, therefore, appeared unrelated to sodium excretion; signs of volume expansion emerged later.

5. Acceleration of the development of spontaneous hypertension by Heymann nephritis, also leading to sustained higher blood pressure levels than in spontaneously hypertensive rats, offers a new approach to experimental study of immune mechanisms behind acceleration of pre-existing hypertension. This may have important bearings on essential hypertension as well.

Key words: hypertension, nephritis, spontaneously hypertensive rat.

Introduction

Hypertension may complicate all forms of glomerulonephritis (Kincaid-Smith, 1977). The relationship between essential hypertension and chronic glomerulonephritis is, however, poorly understood (Mendlowitz, 1979). We have introduced a new model of hypertension that consists of Heymann nephritis (autologous immune complex nephritis) combined with DOCA–NaCl treatment (Tikkanen, Fyhrquist, Miettinen & Törnroth, 1980). In this type of hypertension nephritis was shown to contribute to the development of hypertension, the mechanism of which remained unclear.

In normotensive Wistar rats Heymann nephritis does not elevate blood pressure (Heymann, Hackel, Harwood, Wilson & Hunter, 1959; Bernard, Alexander, Couser & Levinsky, 1978; Tikkanen et al., 1980). It was therefore of interest to study whether such nephritis affects the course of spontaneous hypertension in rats, which is considered to be similar to essential hypertension in man (Grollman, 1972; Folkow & Hallbäck, 1977).

Methods

Animals and blood pressure recordings

Female Wistar rats (24) of the Okamoto–Aoki strain (Møllegaard Hansen, Ejby, Denmark),
obtained when 4 weeks old, were used. Three animals were housed in each cage, kept under 12 h of dark and light variation, and allowed standard rat food, containing 117 mmol/kg of sodium and 235 mmol/kg of potassium, and tap water ad libitum. Systolic blood pressure was recorded in conscious animals between 12.00 and 18.00 hours by a tail-cuff (Harward Apparatus Co., MA, U.S.A.) method with a Doppler ultrasonic flowmeter (Parks Electronics Lab., Beaverton, U.S.A.) to detect the pulse in the tail (Buñag, 1973), which was first warmed for 10 min at 37°C.

Induction of nephritis

At 10 weeks of age, half of the rats were immunized with purified rat kidney brush-border fraction, isolated as described by Miettinen & Linder (1976), to induce autologous immune complex nephritis. The immunization schedule, described in detail elsewhere (Tikkanen et al., 1980), consists of two injections of brush-border fraction in adjuvant at 4 week intervals. Immunization with adjuvant only neither caused proteinuria nor accelerated the development of hypertension in 10 other spontaneously hypertensive rats studied.

Analytical methods

Twenty-four hour urine collections, with measurements of fluid consumption, were made individually from rats in metabolic cages with free access to water but not to food. Seven and 12 weeks after the first immunization 2 ml blood samples were taken from the jugular vein under light ether anaesthesia. The final blood sample was drawn from the abdominal aorta at 17 weeks. EDTA plasma (6 g of disodium EDTA/l of blood) and serum were separated by centrifugation and stored at -20°C until assayed.

Assay of urinary and serum proteins was made as described (Tikkanen et al., 1980). Serum and urinary sodium and potassium were measured by flame photometry (Corning-EEL, Essex, U.K.). Serum and urine creatinine concentrations were determined by a modification (Harjanne, Koivula, Pitkanen & Rauta-Aro, 1977) of the kinetic creatinine assay (Merckotest 3384, Merck) and creatinine clearance was calculated. Urine and serum osmolalities were measured by a vapour pressure osmometer (Wescor Inc., Logan, U.S.A.). Plasma renin activity was determined by radioimmunoassay (Fyhrquist, Soveri, Puutula & Stenman, 1976) modified for rat plasma by using hydroxyquinoline (5·0 mmol/l) as an enzyme inhibitor and pH 6·5 during the angiotensin I generation step. For packed cell volume determination whole blood was centrifuged in heparinized capillary tubes for 6 min at 12 000 g.

Tissue studies

Kidneys and hearts were rapidly removed at the end of the experiment and processed for histological, immunohistological and electron microscopic studies as described (Tikkanen et al., 1980). Hearts were weighed immediately after the rats were killed.

Statistics

Statistical evaluations were performed by Student’s t-test for non-paired observations, preceded by logarithmic transformation for values showing skewed distribution.

Results

Blood pressure and heart weight

Control spontaneously hypertensive rats developed established hypertension at the age of 14 weeks, blood pressure averaging nearly 170 mmHg (Fig. 1). Blood pressure rose to a
Table 1. Heart weight, packed cell volume, creatinine clearance, serum total protein, sodium, potassium, osmolality and plasma renin activity in spontaneously hypertensive rats and in spontaneously hypertensive rats with Heymann nephritis.

Values are means ± SEM at 17 weeks after the first immunization except plasma renin activity also at 7 and 12 weeks. Significance of differences: * P < 0·05; ** P < 0·01; *** P < 0·001 compared with spontaneously hypertensive rats.

<table>
<thead>
<tr>
<th>Analytical variables</th>
<th>Spontaneously hypertensive rats (n = 12)</th>
<th>Spontaneously hypertensive rats with nephritis (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart wt./body wt. (mg/g)</td>
<td>4·64 ± 0·09</td>
<td>5·30 ± 0·09***</td>
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<tr>
<td>Packed cell volume (%)</td>
<td>46·3 ± 0·44</td>
<td>42·9 ± 1·32*</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>1·03 ± 0·07</td>
<td>1·10 ± 0·10</td>
</tr>
<tr>
<td>Serum total protein (g/l)</td>
<td>77·4 ± 0·40</td>
<td>72·4 ± 1·20***</td>
</tr>
<tr>
<td>Serum sodium (mmol/l)</td>
<td>144 ± 0·9</td>
<td>144 ± 2·6</td>
</tr>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>5·3 ± 0·2</td>
<td>5·6 ± 0·2</td>
</tr>
<tr>
<td>Serum osmolality (mosmol/kg)</td>
<td>308 ± 0·8</td>
<td>307 ± 1·1</td>
</tr>
<tr>
<td>Plasma renin activity (ng h⁻¹ ml⁻¹)</td>
<td>8·8 ± 0·5</td>
<td>8·4 ± 0·8 (n = 10)</td>
</tr>
<tr>
<td>7 weeks</td>
<td>8·2 ± 0·6</td>
<td>6·8 ± 0·9 (n = 10)</td>
</tr>
<tr>
<td>12 weeks</td>
<td>9·3 ± 0·6</td>
<td>5·3 ± 1·0**</td>
</tr>
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Significantly higher level in the immunized spontaneously hypertensive rats by 4 weeks after the first immunization (P < 0·05) and remained above 180 mmHg throughout the study (P < 0·01 to P < 0·001, except at 13 weeks, compared with that of the controls). Heart weight was also significantly (P < 0·001) higher in the immunized rats than in the controls (Table 1). Four of the immunized rats died at 4, 7, 14 and 17 weeks respectively, after previous weight loss and generalized wasting. None of the control rats died.

Analytical variables

The daily urinary protein excretion never exceeded 2 mg in the control rats. All immunized rats developed an abnormal proteinuria at the end of the experiment (range 44–397 mg/24 h). Increased values were seen from week 6, the first collection period after the first immunization (Fig. 2).

Serum total proteins were decreased (P < 0·001) in the rats with nephritis at the end of the experiment (Table 1).

Weight gain was slightly lower in the immunized rats than in the controls but the difference was not significant (Fig. 3). Fluid intake and urine volumes increased more in the immunized rats than in the controls (P < 0·05 and <0·01 at 17 weeks respectively). This was accompanied by a decrease in urinary osmolality of the immunized rats (P < 0·001). The immunized rats excreted more potassium than the controls throughout the time after immunization (P < 0·01–0·001). Sodium excretion, however, changed in a biphasic pattern. At 6 weeks, sodium excretion was increased in the immunized rats (P < 0·05 compared with that in the controls) but then decreased towards the end of the experiment (P < 0·01). In the control rats electrolyte excretion remained fairly constant (Fig. 3).

No differences in serum potassium and sodium concentrations, serum osmolality or creatinine clearance were observed between the two groups at the end of the experiment (Table 1). Packed cell volume was lower in the immunized rats (P < 0·05, Table 1). A suppression of plasma renin activity was noted in the immunized rats towards the end of the experiment (P < 0·01 compared with that in the controls at 17 weeks, Table 1). Ascites or oedema was not observed.

Tissue studies

Ten of the 12 immunized rats showed light microscopic changes typical of membranous glomerulonephritis and nine had mild or moderate renal tubular atrophy and/or infiltration of the renal interstitium by mononuclear inflammatory cells. Renal or cardiac vascular changes or glomerular sclerotic changes were not observed. Kidneys and heart from control spontaneously hypertensive rats were histologically normal.
According to the present study, spontaneous hypertension is aggravated when Heymann nephritis (autologous immune complex nephritis) (Heymann et al., 1959; Edgington, Glassock & Dixon, 1967) is induced in spontaneously hypertensive rats during their early life. Heart weights were greater and life span appeared shorter in nephritic rats compared with that in the control spontaneously hypertensive rats. Heymann nephritis per se, however, neither increased blood pressure nor heart weight in normotensive Wistar rats (Tikkanen et al., 1980). Thus the present data demonstrate an additive effect of Heymann nephritis to the raised blood pressure of spontaneously hypertensive rats. An analogous observation was made in DOCA-NaCl treated Wistar rats, in which Heymann nephritis resulted in hypertension, whereas DOCA-NaCl load alone caused only a slight elevation of blood pressure (Tikkanen et al., 1980).

A typical membranous glomerulonephritis and proteinuria developed in the immunized spontaneously hypertensive rats, and interstitial and tubular damage in many of them, as observed also in other rat strains (Alousi, Post & Heymann, 1969; Klassen, Sugisaki, Milgrom & McCluskey, 1971; Allison, Wilson & Gottschalk, 1974; Tikkanen et al., 1980). Thus qualitative features of Heymann nephritis, possibly altered because of genetic properties of spontaneously hypertensive rats, appear similar in these rat strains. Moreover, proteinuria in nephritic spontaneously hypertensive rats corresponded quantitatively to that in nephritic DOCA-NaCl hypertensive rats (Tikkanen et al., 1980).

No renal or cardiovascular lesions were observed in the present study, which is probably explained by the short time of observation (Matthiesen & Tuch, 1978). The proliferative sclerotic glomerular changes of Heymann nephritis...
nephritis–DOCA–NaCl hypertensive rats (Tikkanen et al., 1980) were also lacking. The increased heart weight in the nephritic spontaneously hypertensive rats, as compared with that in the controls, indicates a significant haemodynamic load added to the cardiovascular system by Heymann nephritis in these rats as well.

Heymann nephritis does not lead to azotaemia, at least over a short period (Allison et al., 1974; Tikkanen et al., 1980). Creatinine clearance did not decrease in any of the nephritic spontaneously hypertensive rats. Thus renal failure does not explain the blood pressure rise in these rats. The aggravated hypertension seems not to be renin–angiotensin-dependent either (Table 1).

Fluid turnover was accelerated and both sodium and potassium excretions were increased at 6 weeks after the first immunization. Yet, fluid retention could not be found in the hypertensive rats as judged from the body weights and lack of ascites and oedema. This suggests that sodium excretory capacity is not impaired during the early phase of spontaneous hypertension aggravated by Heymann nephritis. The observed diuresis and natriuresis is likely to be caused by increased perfusion pressure (Guyton, Coleman, Cowley, Scheel, Manning & Norman, 1972), although other factors, such as primary changes in fluid intake, cannot be ruled out. The interpretation of electrolyte data is limited because of lack of observations during the time of diverging blood-pressure rise in the two groups and lacking monitoring of food intake. It is noteworthy, however, that the difference in blood pressure was already established when proteinuria in the rats with nephritis was still minimal.

Sodium retention has been reported during the nephrotic phase of Heymann nephritis (Bernard et al., 1978). Although such a nephrotic syndrome did not develop in the present study, sodium excretion tended to decrease with progression of nephritis in spontaneously hypertensive rats. Excess fluid volume is thought to be of importance in the genesis of hypertension in chronic renal disease (Brown, Fraser, Lever, Morton, Robertson & Schalekamp, 1977). A decrease in packed cell volume, serum proteins and plasma renin activity in the nephritic spontaneously hypertensive rats at the end of the experiment may reflect volume expansion. Further studies on electrolyte and fluid balance, including haemodynamic measurements, are required to clarify a possible relationship between volume factors and hypertension in nephritic spontaneously hypertensive rats.

The mechanism by which the hypertensive effect of Heymann nephritis is mediated appears difficult to determine. The nature of Heymann nephritis itself is still unclear. In addition to the autologous immune complex pathogenesis suggested earlier (Edgington et al., 1967) there is increasing evidence that immune complexes are indeed formed locally in the glomeruli (Couper & Salant, 1980). The effect of Heymann nephritis on the development of hypertension differs from the effect of glomerular injury caused by non-immune mechanisms because chronic \( N,N' \)-diacetylbenzidine-induced nephropathy (Zimmerman, 1979) did not accelerate the development of DOCA–NaCl hypertension in Wistar rats (I., Tikkanen, unpublished work). Immunological mechanisms have been implicated in the course of experimental and human hypertension (Mathews, Whittingham & Mackay, 1974; Editorial, 1978; Svendsen, 1978; Chatelain, Vessey & Ferrario, 1980). Thus the role of the immune system in the pathogenesis of accelerated hypertension due to Heymann nephritis should be further clarified.

Spontaneous hypertension of the rat shares common features with essential hypertension in man (Grollman, 1972; Folkow & Hallbäck, 1977). The present observation on aggravation of such genetic hypertension by Heymann nephritis may have important bearings on human hypertension as well.

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References


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