Biphasic changes in thymus structure during evolving renal hypertension

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Summary

1. Structural changes in the thymus during the evolution of experimental renal hypertension were investigated to determine their possible role in the genesis of hypertensive vascular disease.

2. The thymus, adrenal glands and the progression of hypertensive vascular lesions were investigated in rats during the first 30 days after occlusion of the aorta between the two renal arteries.

3. Hypertension was initially accompanied by marked atrophy of the thymus, most pronounced 9 days after operation. During this time, the adrenal glands doubled in size and the heart became enlarged.

4. After 21 days the thymus regenerated and became hypertrophic. Histological features of hyperactivity accompanied by infiltration of plasma cells were evident, while the adrenal glands remained enlarged.

5. The observed structural changes of the regenerated thymus in the presence of sustained adrenal hypertrophy indicate that the thymus may contribute to the production of hypertensive vascular disease.

Key words: autoimmunity, hypertensive vascular disease, renal hypertension, thymus.

Introduction

Several lines of evidence suggest that immunological factors may contribute to the pathogenesis of hypertensive vascular disease. As indicated by a series of studies, the process appears to affect the humoral and cellular components of the immune system. Autoantibodies directed against kidney and arterial tissue have been described by White & Grollman (1964), while Olsen (1973) and Olsen & Loft (1973) have demonstrated that lymphocytes obtained from hypertensive donors appear to be directed against vascular antigens. Rojo-Ortega, Yeghiayan & Genest (1973) have shown that the thymuses of spontaneously hypertensive rats have histological abnormalities, which suggest auto-aggression as a part of the disease process. These findings are reinforced by experiments in which partial infarction of a kidney results in significantly lesser vascular disease and hypertension in rodents with genetic aplasia of the thymus than in hypertensive control animals with normal thymuses (Svendsen, 1976, 1977).

Furthermore, the transfer of lymphocytes from hypertensive donors to normotensive recipients appears to enhance the development of vascular disease (Olsen, 1971; Svendsen, 1973) or hypertension (Okuda & Grollman, 1967). Since the thymus is considered a capital organ in the evolution of autoimmune processes (Burnet, 1962; Miller, 1963), we investigated the morphological changes in the rat thymus during the evolution of experimental renal hypertension as a first step in characterizing its possible role in this disease.
Materials and methods

Outbred male Sprague–Dawley rats (128, 42–50 days of age) were rendered hypertensive by total occlusion of the abdominal aorta between the two renal arteries as described by Rojo-Ortega & Genest (1968). Fifty-six other sham-operated rats served as control animals. Animals were mixed in cages at random to avoid any behavioural differences resulting from the surgical procedure. In previous experiments (unpublished observations) it had been determined that the thymus in normal rats of this strain reaches its highest weight around the day 56 of age (230 g of body weight). Thus animals with an initial weight between 150 and 200 g were used in the present study.

Groups of 12 hypertensive rats were studied at intervals of 3 days between 3 and 30 days after aortic ligation. The results were compared with those obtained in groups of eight age-matched control rats studied between 3 and 30 days after sham operation. On the day of the experiments, a catheter (PE-50, Clay Adams, N.J., U.S.A.) was inserted into a carotid artery under light ether anaesthesia. Arterial blood pressure was recorded after 6 h, when the animals had recovered from the anaesthesia. Measurements were obtained with the rats mildly restrained in a plastic box. After arterial pressure had been recorded for 1 h, the animals were killed rapidly; their kidneys, heart, thymus, spleen and adrenal glands were dissected free, blotted dry and weighed. Sections (2 mm) from these organs and, in addition, tissue samples from mesentery, liver and pancreas, were fixed in either ethanol or 10% formalin. Sections from tissues embedded in paraffin were cut 4–5 μm thick and stained with haematoxylin/eosin/azure II, or Methyl Green/pyronin, or periodic acid/Schiff reagent or Lillie’s allochrome stains. Sections from tissues embedded in plastic (Araldite 502, R.P. Cargille Laboratories, Cedar Grove, N.J., U.S.A.) were cut 1 μm thick and stained with 1% Toluidine Blue. Frozen sections from the adrenal glands were stained with Sudan IV. The width of the zona glomerulosa was measured on sections stained with haematoxylin/eosin/azure II. Camera lucida outlines of the glomerulosa were made at four different points around the gland at a magnification of ×500. Readings were averaged and converted into μm.

Results

Three days after aortic ligation, the mean blood pressure of renal hypertensive rats rose to 163 ± 2 (SE) mmHg compared with 122 ± 3 mmHg in eight sham-operated control rats (P < 0.001). Blood pressures reached a plateau at about 15 days after operation; at this time it averaged 191 ± 3 mmHg (Fig. 1). Blood pressure in sham-operated control animals did not change significantly (Fig. 1). Onset of hypertension was accompanied by cardiac hypertrophy, adrenal gland enlargement and variable changes in spleen weight (Table 1, Fig. 1). The left kidney (below the ligature) became frankly atrophic due to ischaemia and there was a compensatory increase in the size of the right kidney (Table 1).

During the first 9 days after operation, body weight declined to about 75% of control; it gradually returned to control values 12–15 days after operation (Table 1). At 3 and 21 days necrotizing arteriolitis, plasma insudation and fibrinoid deposition were observed in vessels of the mesenteric territory, pancreas and right kidney. In contrast, after 21 days pathological features resembling proliferative endarteritis and periarteritis

![Figure 1](image-url)
Thymus in renal hypertension

Table 1. Body weight and relative weight of the organs in hypertensive and sham-operated rats

Mean values ± 1SEM are shown. n.s., Not significant. P refers to significance of differences between control and sham-operated animals by unpaired t-test.

<table>
<thead>
<tr>
<th></th>
<th>Body wt. (g)</th>
<th>Relative organ weight (mg/100 g body wt.)</th>
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<tbody>
<tr>
<td></td>
<td>Heart</td>
<td>Right kidney</td>
</tr>
<tr>
<td>3 days</td>
<td></td>
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<tr>
<td>Hypertensive</td>
<td>145.5 ± 9</td>
<td>445 ± 10</td>
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<tr>
<td>Sham-operated</td>
<td>164.2 ± 4</td>
<td>312 ± 5</td>
</tr>
<tr>
<td>P</td>
<td>n.s.</td>
<td>&lt;0.001</td>
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<tr>
<td>9 days</td>
<td></td>
<td></td>
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<tr>
<td>Hypertensive</td>
<td>130.6 ± 22</td>
<td>487 ± 12</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>227.3 ± 5</td>
<td>311 ± 9</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
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<tr>
<td>15 days</td>
<td></td>
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<tr>
<td>Hypertensive</td>
<td>147.8 ± 11</td>
<td>468 ± 16</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>249.3 ± 7</td>
<td>319 ± 8</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>21 days</td>
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<td></td>
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<tr>
<td>Hypertensive</td>
<td>195.9 ± 46</td>
<td>441 ± 19</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>289.3 ± 11</td>
<td>306 ± 15</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>30 days</td>
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<tr>
<td>Hypertensive</td>
<td>257.2 ± 27</td>
<td>424 ± 19</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>348.7 ± 12</td>
<td>306 ± 13</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.025</td>
<td>&lt;0.001</td>
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nodosa were observed in the mesentery, right kidney, pancreas, heart and intestine. Neither acute nor chronic vascular lesions were encountered in the thymus gland of hypertensive animals.

Development of experimental hypertension was accompanied by striking changes in the structure and size of the thymus (Fig. 2 and Fig. 3). A marked decrease in the weight of this organ was observed 3 days after operation (Fig. 1), and the lowest weight was recorded 9 days after onset of hypertension. This coincided with the time at which thymuses from sham-operated animals of the same age had the greatest weight (Fig. 1). Under light microscopy, thymus atrophy appeared to be due to marked depletion of cortical lymphocytes (Fig. 3). Various stages of cell degeneration were evident; there was pyknosis and karyorrhexis and an increase in the number of macrophages, which were filled with nuclear debris. A lesser degree of lymphocytic depletion was observed in the medulla. This resulted in an apparent reversal of the normal pattern of the cortex and medulla (Fig. 2 and Fig. 3). The diminution in the population of lymphocytes from the thymic cortex uncovered a meshwork of enlarged epithelial reticular elements (Fig. 3). These cells showed signs of hypertrophy, structural features of hypersecretion and an increase in periodic acid/Schiff, positive inclusions in both cortex and medulla. These changes contrasted sharply with the normal appearance of the thymus examined in sham-operated animals (Fig. 2). Thymus atrophy was accompanied by a twofold increase in adrenal weight (Fig. 1). Nine days after onset of renal hypertension, the glomerulosa zone increased in size to 102 ± 7 μm compared with 44 ± 3 μm (P < 0.05) in sham-operated control rats. Both hypertrophy and hyperplasia appeared to account for the enlarged zona glomerulosa and numerous mitoses were observed. The fasciculata zone was also slightly increased in size. Considerable dilatation of the sinusoids due to hyperaemia was evident in this region. Vascular lesions or plasma insudation were not observed in the adrenal parenchyma although hypertrophied arterioles and fibrinoid deposition were occasionally present in the capsule of the gland.

The period between 12 and 21 days after onset of hypertension was characterized by structural recovery of the thymus gland. However, disparity with the control rats was still remarkable, and a
FIG. 2. Section of thymus from a normotensive sham-operated control rat. A normal cortex–medulla pattern is observed. (Plastic-embedded section, Toluidine Blue stain, x160.)

FIG. 3. Section from the thymus of a 9 day hypertensive rat. Cellular derangement accompanying thymus atrophy. The normal line of demarcation between cortex and medulla is lost. Cellular depletion is observed in the cortex. The medulla appears to be less severely depleted. Enlarged epithelial reticular cells can be observed as a confluent meshwork in the cortex. (Lillie allochrome stain, x200.)
Wide variety of reconstitutive stages were observed. Marked lymphoblastic proliferation, high lymphocyte mitotic activity without recovery of the original pattern of cortex and medulla, and formation of germinal follicles in the periphery of the lobules were predominant findings. After 24 days thymic hypertrophy was evident. The cortex was now repopulated by lymphocytes and there was a significant increase in its size when compared with the sham-operated controls (Fig. 1). At this time there was an increase in the macrophagic activity of this region. In the medulla Hassall's corpuscles and epithelial cysts filled with colloid were abundant. The reticuloepithelial cells remained enlarged, but were now separated by a growing number of lymphocytes. Plasma cells, which had not been observed until this time, were evident when stained with Methyl Green/pyronin, periodic acid/Schiff or azure II stains (Fig. 4). These cells were never found in thymus obtained from sham-operated control rats. In keeping with the general increase in glandular activity there was a marked increase in the vascularity of the thymus; structures resembling 'germinal centres' were occasionally present in the medulla (Fig. 5).

Discussion

Abnormal accumulation of plasma cells and marked hyperplasia of reticuloepithelial cells with structural features of hypersecretory activity have previously been observed by Rojo-Ortega et al. (1973) in the thymus of aged spontaneously hypertensive rats with severe vascular lesions and prolonged hypertension. Our study has shown that similar changes occur in rats with renal hypertension, whereas previously there has been little reason to suspect that immunological alterations may be involved in the pathogenesis of hypertension. This stage of apparent hyperactivity appears to follow an early phase of severe thymus atrophy.

The biphasic response of the thymus during the evolution of severe renal hypertension was accompanied by a persistent enlargement in the weight of the adrenal gland. These findings are at variance with what is known about a possible endocrine counterbalance between these two organs (Soffer, Dorfman & Gabrilove, 1961). It has been shown that adrenalectomy results in thymic enlargement (Jaffe, 1924), and adrenal hypertrophy follows...
thymectomy (Soli, 1909). Administration of corticosteroids produces a strong involution of all lymphatic tissue (Dougherty, 1952), and hypoplasia of the adrenal glands can be induced by the administration of thymus extracts (Rowntree, 1935). These experimental findings are supported by studies of human pathology. For example, atrophy of the adrenal gland in Addison's disease is accompanied by lymphoid hyperplasia. A persistently enlarged thymus and adrenal gland atrophy are found in Graves' disease (Boyd, 1947). Increased concentrations of circulating corticoids may be accompanied by a reduction in thymus size (Dougherty, 1952). The inverse relationship between these systems is illustrated by the fact that irradiation, cyclophosphamide or folic acid antagonists can produce atrophy of the thymus gland but hypertrophy of the adrenal gland (Gardner, Quagliata, Drossman, Kalish & Schimmer, 1970; Dougherty, 1952). We are not certain whether or not the marked involution of the thymus during the early phase of renal hypertension results from increased amounts of circulating corticoids, but the increase in adrenal weight, and in its histological hypersecretory features, is suggestive evidence for the former. Slightly increased concentrations of mineralo- and gluco-corticoids have also been reported in the chronic phase of hypertension in the Sprague-Dawley rat (Singer, Losito & Salmon, 1963; Dietz, Mast, Mohring, Vecsey, Glass, Oster & Gross, 1975).

The pronounced atrophy of the thymus may produce an immunological imbalance which can be responsible for further vascular damage. Animals with immune defects and patients treated with irradiation, alkylating or immunosuppressive agents often have associated autoimmune abnormalities and vascular and connective tissue diseases (Miller & Osoba, 1967; Schwartz, 1965; Waller, Irby, Mullinaux & Toone, 1965). Hypergamma-globulinaemia (Ebringer & Doyle, 1970), increased rheumatoid serum factor (Mathews, Whittingham, Hooper & Mackay, 1973), and haemolytic anaemia (apparently not of the autoimmune type)
have been reported in a large number of hypertensive patients (Heptinstall, 1974).

In our study, the secondary increase in thymus weight was a result of dysplasia rather than hyperplasia. Enlarged reticuloepithelial cells and an increased number of Hassall's corpuscles were found, along with an altered cortex/medulla ratio. The presence of lymphoid follicles and plasma cells (the latter also described in the thymus of spontaneously hypertensive rats) are pathological features strongly suggestive of an autoimmune process (Burnet, 1962, 1971). In man, hyper trophy of the thymus is mostly due to increased reticuloepithelial cell activity and an increased number of Hassall's corpuscles (Mackay, 1966). These features have been reported in thyrotoxicosis or Hashimoto's thyroiditis where autoimmune disturbances are implicated in their pathogenesis (Mackay, 1966; Irvine, 1966). Lymphoid follicles and plasma cells are also characteristic of myasthenia gravis (Mackay & Burnet, 1966) and lupus erythematosus (Mackay & DeGail, 1963). These processes are widely recognized to be auto-aggressive in nature. We do not yet know the significance of the structural changes of the thymus during the development of hypertension. If it were to be the reflection of or the cause of an immunological imbalance, it could play a contributory role in the development of vascular disease. Finally, Wexler, Iams & Judd (1976) have observed the triad of adrenal gland hypertrophy, thymus enlargement and vascular disease in repeatedly bred Sprague–Dawley rats. Periarteritis nodosa, thrombotic thrombocytopenic purpura, amyloidosis, scleroderma and lupus erythematosus, widely held to be autoimmune diseases (Mackay & Burnet, 1966), present high blood pressure as a common symptom in the secondary stage (Winter, 1964; Heptinstall, 1974). These findings may suggest that the immune system is somehow involved in the development of extensive vascular and renal damage which could contribute to the maintenance of increased blood pressure.

The changes in size and structure of the thymus in experimental hypertension suggest a role of this organ in the pathogenesis of hypertensive vascular disease.

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References


