Pathophysiology of Aortic Baroreceptors in Rabbits with Vitamin D Sclerosis and Hypertension

By Jennifer E. Angell-James

ABSTRACT

Calciferol (vitamin D₂) (50,000–100,000 IU) and calcium lactate (1 g) were added to the normal diet of 22 rabbits for 7–11 days. When mean arterial blood pressure had risen from a control value of 87.2 ± 3.7 mm Hg to an experimental value of 137.8 ± 5.7 mm Hg (11–145 weeks), the aortic arch of 9 of these rabbits was isolated and perfused with Krebs-Henseleit solution. The impulse activity in 75 aortic baroreceptor fibers from the left aortic nerve was studied during nonpulsatile perfusion at different pressures and was compared with the impulse activity in 29 fibers from 17 normal rabbits. The threshold pressures and the pressure at the point of inflection of the curves were lower in these fibers than they were with fibers from normal rabbits. The gradient of the curves relating baroreceptor impulse frequency to aortic pressure was depressed from a normal mean value of 1.19 impulses/sec mm Hg⁻¹ to 0.61 impulses/sec mm Hg⁻¹ (P < 0.001). A similar depression of the gradient was found in 7 baroreceptor fibers from the right aortic nerve. The change in the sensitivity of the baroreceptors was more closely related to the time interval after the calciferol treatment (r = −0.95) than it was to the mean arterial blood pressure (r = −0.76). The pressure-volume curves show that it was also related to the decreased distensibility of the aortic arch region. Histologically, the aortic arch region had extensive medial sclerosis with calcification.

KEY WORDS
heart aortic pressure-volume curves baroreceptor pathology kidney baroreceptor activity aorta pathology

The activity of arterial baroreceptors is modified in experimental renal hypertension (1–4) and in experimental atherosclerosis (5) that is associated with mild hypertension.

Hypertension has also been reported in humans with severe idiopathic hypercalcemia (6), but no studies of baroreceptor activity in arterial disease involving extensive sclerosis of the media have previously been reported.

In the present paper baroreceptor activity in rabbits with experimentally induced medial sclerosis is described. An isolated, perfused aortic arch preparation was used to study baroreceptor function under controlled conditions. The characteristics of the baroreceptors from the rabbits with medial sclerosis were compared with those from normal rabbits undergoing similar experimental procedures (7) and were correlated with alterations in the pathology and distensibility of the aortic arch.

Methods

PRODUCTION OF MEDIAL SCLEROSIS

A total of 22 New Zealand White rabbits weighing 1.4–2.6 kg were given a diet of S.C.I pellets for up to 145 weeks. Their diet was supplemented with calciferol (vitamin D₂) (50,000–100,000 IU) and calcium lactate (1 g) administered orally for 7–11 days at the beginning of this period.

Serum calcium, phosphate, alkaline phosphatase, and cholesterol were measured during the control period before the administration of the diet and on the tenth day during the administration of calciferol and calcium lactate. The results are summarized in Table 1.

The mean blood pressure of the unanesthetized rabbits was measured before the administration of the diet and thereafter at weekly intervals with a Grant-Rothschild capsule placed over the central ear artery (8).

TERMINAL EXPERIMENT

A total of 11 rabbits survived until the terminal experiment took place 11–145 weeks after the commencement of the special diet. In 9 rabbits the mean blood pressure was measured with an electromanometer connected to a polyethylene cannula that was inserted into the central artery of the ear under local anesthesia. The signals were recorded after suitable amplification on a multichannel ultraviolet light-recording oscillograph. In
TABLE 1
Serum Electrolyte and Cholesterol Levels in the Calciferol-Treated Rabbits before Treatment and on the Tenth Day of Treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Tenth day</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/100 ml)</td>
<td>13.8 ± 0.16</td>
<td>13.1 ± 0.31</td>
<td>-0.65 ± 0.31</td>
</tr>
<tr>
<td>Phosphate (mg/100 ml)</td>
<td>6.3 ± 0.12</td>
<td>7.6 ± 0.39</td>
<td>1.3 ± 0.43</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/100 ml)</td>
<td>13.57 ± 1.2</td>
<td>8.14 ± 0.75</td>
<td>-5.4 ± 1.21</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>54.3 ± 9.5</td>
<td>133.6 ± 22.9</td>
<td>73.2 ± 6.3</td>
</tr>
</tbody>
</table>

these rabbits the mean pressure, which was obtained electrically, was 121.3 ± 8.2 mm Hg.
The 11 calciferol-treated rabbits used in the terminal experiment weighed 3.5 ± 0.26 kg and were anesthetized with urethane (1.6 ± 0.04 g/kg, iv). A tracheostomy was performed, and a cannula (1.6 mm, o.d.) was passed down the common carotid artery until its tip lay in the aortic arch. Heparin (1,000 IU/kg, ia) was also administered.

ISOLATION AND PERFUSION OF THE AORTIC ARCH

The aortic arch was isolated from the rest of the circulation as previously described (7, 9) by ligating all the branches arising from it (10) except the right common carotid artery which was cannulated. The aortic arch was perfused with Krebs-Henseleit solution by a cannula that was inserted into the ascending aorta through the wall of the left ventricle. The descending thoracic aorta was ligated at the level of the left pulmonary artery. The temperature of the perfusate was measured with a thermocouple in the tip of the aortic cannula and was maintained at 37-39°C. The aortic pressure was measured by an electromanometer via a cannula; the tip of the cannula was in the ascending aorta.

Single- and few-fiber recordings were made from the left and the right aortic nerves using silver-silver chloride electrodes, and the recordings were made on ultraviolet light-sensitive paper after suitable amplification. Full details of this technique have been described previously (7, 9).

METHODS OF ANALYSIS OF RESULTS

The method of analysis of the single-fiber activity in this study was the same as that reported previously (4, 7). The impulse frequency was plotted against the aortic arch pressure. The lowest pressure at which the baroreceptors discharged any impulses was called the threshold pressure. Above the threshold there was usually a linear relationship between the pressure and the impulse frequency, and the gradient of this part of the curve was measured. The point at which this linear relationship ceased was called the point of inflection. The threshold index and the index at the point of inflection were calculated by dividing the impulse frequency at these points by the pressure and expressing them as impulses/sec mm Hg⁻¹.

STATISTICAL ANALYSIS

The arithmetic mean values ± SE for a sample were calculated. To test the significance of the difference of the means of two grouped values, t was calculated as the difference of the means divided by the standard error of the difference of the means. The probability P, corresponding to the number of degrees of freedom N = n₁ + n₂ - 2, was found from tables.

The relationships of the baroreceptor activity to the arterial blood pressure and to the interval of time from the commencement of the calciferol diet were estimated by calculating the regression line, which was weighted to include the number of fibers in each mean value.

PRESSURE-VOLUME CURVES

Pressure-volume curves of the aortic arch were determined by measuring the peak pressure obtained in the perfused area after the injection of a known volume of Krebs-Henseleit solution at 37°C as previously described (4). The peak pressure was plotted against the injected volume expressed as a percent of the original volume.

HISTOLOGICAL STUDIES

Tissues were removed from the carotid sinuses, the ascending and the descending thoracic aorta, the heart, the kidney, and the right subclavian artery. The specimens were fixed in 10% Formalin-saline solution and embedded in wax. The histological sections were stained with hematoxylin and eosin, Verhoeff and Van Gieson, Von Kossa, toluidine blue, Masson, and Gomori stains. Histochemical studies were made on the left carotid sinus and the middle of the aortic arch by a modification of the Koelle technique (11).

Results

Ten days after the calciferol and calcium diet was begun, serum calcium level of the rabbits was slightly lower than that during the control period, and the serum phosphate level was slightly higher. The alkaline phosphatase level was also marginally lower (Table 1), and there was a significant elevation in the serum cholesterol level from a mean control value of 54.3 mg/100 ml to 133.6 mg/100 ml (P < 0.001).

Eleven of the 22 rabbits that were given the calciferol diet died from the toxic effects of the calciferol. The remaining 11 rabbits were killed at the terminal experiment which was carried out...
11-145 weeks after the diet was begun. Mean blood pressure rose from a control value of 84.5 ± 3.5 mm Hg (range 70 to 110 mm Hg) to a peak level of 155.2 ± 8.6 mm Hg (range 120 to 210 mm Hg) 10.1 ± 1.8 weeks after the diet began; it then fell to a value of 126.0 ± 6.9 mm Hg (range 85 to 175 mm Hg) at the time of the terminal experiment. A typical record of the weekly blood pressure readings which indicates that the hypertension began to develop within 2 weeks is shown in Figure 1.

The blood pressure of these hypertensive rabbits was remarkably labile and altered from day to day and from minute to minute. The changes in blood pressure which occurred during small spontaneous movements were measured in the unanesthetized rabbits via a cannula inserted into the central ear artery. The maximum change in the mean blood pressure in five rabbits was 37.0 ± 7.0 mm Hg (range 30 to 65 mm Hg). In normal rabbits the maximum change in the mean blood pressure under similar circumstances was 15 mm Hg.

On the day of the terminal experiment the rabbits were anesthetized, and the aortic pressure was measured by an electromanometer via a catheter inserted through the right common carotid artery. The maximum change in the mean blood pressure in five rabbits was 266.3 ± 18.6 beats/min (range 177 to 360 beats/min), and the heart rate of the normal rabbits was 258.2 ± 19.2 beats/min; the systolic pressure (153.0 ± 13.3 mm Hg) and the diastolic pressure (92.5 ± 10.1 mm Hg) were both higher than those pressures in normal rabbits, and the pulse pressure (60.5 ± 8.5 mm Hg) was also higher than the pulse pressure in normal rabbits (31.0 ± 4.0 mm Hg).

BARORECEPTOR IMPULSE ACTIVITY AND AORTIC ARCH PRESSURE

The aortic arch was successfully isolated from the rest of the circulation and was perfused in situ in 9 of the 11 calciferol-treated rabbits; in the remaining 2 rabbits the vessels were so heavily calcified that they ruptured when the ligatures were tied around them.

Aortic Arch Baroreceptor Fibers.—The mean value for the threshold pressure below which the aortic baroreceptors were inactive was significantly lower than that of normal rabbits studied previously (7) (P < 0.05, Table 2), although the range was similar. Many of the 75 fibers studied had threshold pressures below 20 mm Hg (Fig. 2). Moreover, these fibers fired at impulse frequencies similar to those of normal fibers at their threshold pressure (P > 0.3), although this pressure was lower, causing a significant increase in the
threshold index of the calciferol-treated rabbits ($P < 0.05$).

The types of discharge at the threshold pressure in the calciferol-treated rabbits were not dissimilar from those of the normal rabbits; 69% of the calciferol-treated rabbits had a critical type 1 threshold pressure compared with 65.5% of the normal rabbits. A total of 11% of the calciferol-treated rabbits had a plateau (type 3) threshold compared with 20.7% of the normal rabbits; the impulse frequency remained constant over a range of pressure before it increased with further increments in pressure. An intermittent (type 2) discharge at the threshold pressure occurred in a larger number of fibers from the calciferol-treated rabbits (20%); only 13.6% of the fibers from normal rabbits showed type 2 discharge.

As the aortic arch pressure was raised above the threshold pressure, the impulse frequency usually increased linearly until the point of inflection was reached. An example of a typical record is illustrated in Figure 3. In comparison with the values for normal rabbits (7), there was a reduction in both the pressure at which the point of inflection occurred ($P < 0.05$) and the impulse frequency at this point ($P > 0.1$, Table 2) for the calciferol-treated rabbits; therefore, the inflection index was no different from that of normal rabbits ($P > 0.8$). The impulse frequency-pressure curves in some of the calciferol-treated rabbits were abnormal in that they were irregular: the impulse frequency increased or decreased rapidly with small elevations in pressure. Occasionally these curves exhibited two separate points of inflection at different pressures. Considering the mean values for the individual experiments, it appears that in six rabbits the impulse frequency-pressure curves were shifted to the left and in three rabbits they were shifted to the right of normal curves (Fig. 4). Two rabbits from the latter group (rabbits 4 and 5) had minimal aortic lesions without calcium but lesions were present in their kidneys. The third rabbit in this group (rabbit 7) had fairly extensive aortic calcification that presumably accounted for low point of inflection, although the mean threshold pressure was elevated (Table 3); however, again there was extensive renal damage.

The gradient of the first part of the impulse frequency curve is an index of sensitivity of the aortic baroreceptors and was markedly reduced in the case of the calciferol-treated rabbits.

### Table 2

Comparison of the Characteristics of the Impulse Activity in Single Baroreceptor Fibers of the Left and the Right Aortic Nerves of Normal and Calciferol-Treated Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Calciferol-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of rabbits</strong></td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>2.2±0.12 (1.4–3.4)</td>
<td>3.5±0.3 (2.1–3.3)</td>
</tr>
<tr>
<td><strong>Blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>47.8±3.7 (75–110)</td>
<td>148.9±7.5 (120–175)</td>
</tr>
<tr>
<td>Peak</td>
<td>8.9±2.0 (2–22)</td>
<td>137.8±5.7 (120–175)</td>
</tr>
<tr>
<td>Week</td>
<td>17.3±1.5 (11–22)*</td>
<td></td>
</tr>
<tr>
<td><strong>Threshold</strong></td>
<td>143 (141&amp;145)</td>
<td></td>
</tr>
<tr>
<td><strong>Number of fibers</strong></td>
<td>29</td>
<td>75</td>
</tr>
<tr>
<td><strong>Pressure (mm Hg)</strong></td>
<td>52.5±5.5 (0–118)</td>
<td>37.8±3.8 (0–110)</td>
</tr>
<tr>
<td><strong>Frequency (impulses/sec)</strong></td>
<td>34.1±2.3 (12–59)</td>
<td>31.1±2.1 (2–83)</td>
</tr>
<tr>
<td><strong>Index (impulses/sec mm Hg−1)</strong></td>
<td>2.89±0.21 (0.13–59)</td>
<td>8.61±1.9 (0.03–63)</td>
</tr>
<tr>
<td><strong>Gradient (impulses/sec mm Hg−1)</strong></td>
<td>1.19±0.14 (0.26–2.85)</td>
<td>0.61±0.05 (0.5–1.42)</td>
</tr>
<tr>
<td><strong>Point of inflection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of fibers</strong></td>
<td>19</td>
<td>53</td>
</tr>
<tr>
<td><strong>Pressure (mm Hg)</strong></td>
<td>112.4±6.2 (63–158)</td>
<td>95.0±4.6 (45–155)</td>
</tr>
<tr>
<td><strong>Frequency (impulses/sec)</strong></td>
<td>73.9±5.4 (35–118)</td>
<td>61.9±4.4 (10–171)</td>
</tr>
<tr>
<td><strong>Index (impulses/sec mm Hg−1)</strong></td>
<td>0.67±0.05 (0.22–0.91)</td>
<td>0.69±0.38 (0.08–1.32)</td>
</tr>
</tbody>
</table>

All values are means ± SE. The range is given in parentheses.

*Data from 7 rabbits.

†Data from 2 rabbits.
calciferol-treated rabbits. The gradient of these curves was diminished from a normal mean value of 1.19 impulses/sec mm Hg⁻¹ to 0.61 impulses/sec mm Hg⁻¹ (P < 0.001, Fig. 3 and Table 2). A summary of the results from the individual experiments is shown in Table 3.

**Right Subclavian Baroreceptors.**—Seven fibers from the right aortic nerve of one rabbit (no. 6) were studied when the pressure in the aortic arch was raised in steps. There was a large range in both their threshold pressures and the points of inflection. The most significant difference was the reduction in the gradient of the curves relating impulse frequency to pressure from a normal value for the fibers of the right aortic nerve of 1.13 ± 0.14 impulses/sec mm Hg⁻¹ to 0.59 ± 0.04 impulses/sec mm Hg⁻¹ (P < 0.01) (Table 2).

**EFFECT ON THE BARORECEPTOR IMPULSE FREQUENCY OF FIRST RAISING THE AORTIC ARCH PRESSURE IN STEPS AND THEN REDUCING IT IN STEPS**

The results obtained in calciferol-treated rabbits were compared with those from similar experiments performed on normal rabbits (Table 4). The typical record from one experiment is illustrated by Figure 3.

In both the calciferol-treated rabbits and the normal rabbits the threshold pressures of the baroreceptors were usually higher when the aortic arch pressure was reduced than when the pressure was increased. The difference was significant in the calciferol-treated rabbits (P < 0.05). Furthermore, the impulse frequency was less at equivalent pressures when the pressure was reduced so that a hysteresis loop relating impulse frequency to aortic pressure was formed. The separation of the curves was greater at the lower pressures than it was at the higher pressures; this phenomenon was more pronounced in the calciferol-treated rabbits since there was a significant difference in the impulse frequency at 80 mm Hg above the threshold pressure (P < 0.05), whereas there was no difference in the normal group even at a lower pressure of 60 mm Hg above the original threshold (P > 0.2).

**RELATIONSHIP BETWEEN THE LEVEL OF THE MEAN ARTERIAL BLOOD PRESSURE AND THE BARORECEPTOR IMPULSE FREQUENCY**

In the calciferol-treated rabbits the mean baroreceptor threshold pressure was in general the same or lower than that in normal rabbits, and the mean arterial blood pressure in the intact rabbit at the time of the terminal experiment was higher; however, there was no simple correlation between these two parameters (gradient −0.43 ± 0.15 mm Hg/mm Hg, r = −0.27). Similarly there was no

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>Normal</th>
<th>Calciferol-treated</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right aortic nerve</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 ± 0.20 (1.9-3.0)</td>
<td>2.4</td>
<td></td>
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<tr>
<td></td>
<td>80</td>
<td>175</td>
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<tr>
<td></td>
<td>175</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 0.05</td>
<td>53.2 ± 9.2 (0-104)</td>
<td>74.3 ± 20.45 (20-140)</td>
<td>&gt; 0.3</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.3</td>
<td>33.0 ± 6.0 (7-71)</td>
<td>27.4 ± 4.27 (12-38)</td>
<td>&gt; 0.4</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.05</td>
<td>4.28 ± 3.39 (0.67-34)</td>
<td>0.5 ± 0.77 (0.27-0.8)</td>
<td>&gt; 0.3</td>
</tr>
<tr>
<td></td>
<td>&lt; 0.001</td>
<td>1.13 ± 0.14 (0.58-2.2)</td>
<td>0.59 ± 0.04 (0.14-0.83)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 0.05</td>
<td>97.7 ± 5.5 (80-121)</td>
<td>88.6 ± 28.1 (45-220)</td>
<td>&gt; 0.7</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.1</td>
<td>65.5 ± 5.3 (48-95)</td>
<td>43.6 ± 4.63 (32-60)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>&gt; 0.8</td>
<td>0.7 ± 0.1 (0.4-1.19)</td>
<td>0.53 ± 0.08 (0.23-0.72)</td>
<td>&gt; 0.2</td>
</tr>
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</table>
direct correlation between the pressure of the point of inflection and the mean arterial blood pressure of each individual rabbit (gradient $-0.6 \pm 0.15$ mm Hg/mm Hg, $r = -0.45$), although the point of inflection was lower in seven of the calciferol-treated rabbits than that in normal rabbits. In general, the change in the threshold pressure was more closely related to the time from the beginning of the calciferol treatment than it was to the actual level of the mean arterial blood pressure (gradient $-0.31 \pm 0.04$ weeks/impulses sec$^{-1}$ mm Hg$^{-1}$, $r = -0.65$), but two rabbits had higher mean threshold levels than did normal rabbits.

In all of the calciferol-treated rabbits the gradient of the curves relating impulse frequency to aortic arch pressure was lower than that in normal rabbits, and there was some correlation between the change in the gradient and the level of the mean arterial blood pressure (gradient $-66.3 \pm 5.6$ mm Hg/impulses sec$^{-1}$ mm Hg$^{-1}$, $r = -0.76$). However, the rabbit with the highest mean arterial blood pressure showed only a small change in the gradient of the two baroreceptor fibers studied from the aortic arch region; the seven fibers from the right aortic nerve of this rabbit had a low gradient, as would be expected from this correlation (Table 2).

The depression of the gradient of the impulse frequency-pressure curves was more closely correlated with the length of time from the commencement of the diet (Fig. 5) than it was with the mean arterial blood pressure (gradient $-26.3 \pm 1.0$ weeks/impulses sec$^{-1}$ mm Hg$^{-1}$, $r = -0.95$); it was least in the rabbit that had been kept for the shortest time, although this rabbit had the highest mean arterial blood pressure. Two rabbits were not included in this correlation because they had been kept much longer (141 weeks) than the other rabbits. Nevertheless, both of these rabbits had gradients relating impulse frequency to aortic arch pressure which were lower than those in normal rabbits.

**PRESSURE-VOLUME CURVES**

In nine calciferol-treated rabbits (weight $3.5 \pm 0.3$ kg, mean arterial blood pressure $137.8 \pm 5.7$ mm Hg), ten pressure-volume curves...
of their aortic arch regions were determined and compared with eight pressure-volume curves from a group of four normal rabbits (weight 3.2 ± 0.15 kg, mean arterial blood pressure 83.4 ± 1.3 mm Hg).

The volume of the region under study was larger in the calciferol-treated rabbits (1.34 ± 0.16 ml) than it was in the normal rabbits (0.7 ± 0.07 ml), although both groups had similar weights. This finding indicated that the arteries in the calciferol-treated rabbits were larger than those in the normal rabbits at zero pressure. Furthermore, the arteries in the calciferol-treated group were less distensible than those in the normal group, since the aortic arch pressure rose more steeply at smaller injected volumes. The pressure-volume curve inflected when 125% of the initial volume had been injected, whereas in the normal group this inflection did not occur until 350% of the initial volume had been injected (Fig. 6). Thus the difference in the pressure-volume curves was much more marked at the larger injected volumes. In general, the less distensible the arteries the more severe the degree of the pathological lesions. Hence the least distensible vessels were observed in rabbits 6, 8, and 9 that had gross lesions affecting the large arteries.

**PATHOLOGY**

**Arterial Lesions.**—Macroscopically the arteries from the calciferol-treated rabbits appeared elongated and tortuous in many instances. Frequently, a lattice-work of calcium showed through the walls of the arteries which failed to collapse or retract when excised and, in some instances, were held rigid in a similar position to the one they had in vivo.

Microscopically the arterial walls had gross lesions affecting the media. The most extensive lesions were observed in the ascending aorta and the origins of the subclavian arteries. The carotid sinus regions, the common carotid arteries, the aortic
TABLE 4
Comparison of the Difference (B−A) in the Effect on Aortic Baroreceptor Activity of Reducing Aortic Pressure in Steps (B) after Previously Raising the Pressure in Steps (A) in Normal and Calciferol-Treated Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th></th>
<th>Calciferol-treated</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rabbits</td>
<td>6</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Number of fibers</td>
<td>6</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>85</td>
<td></td>
<td>142.0</td>
<td></td>
</tr>
<tr>
<td>Threshold pressure difference (mm Hg)</td>
<td>12.5 ± 8.9</td>
<td>&gt;0.2</td>
<td>15.9 ± 6.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Threshold frequency difference (impulses/sec)</td>
<td>-2.3 ± 3.3</td>
<td>&gt;0.5</td>
<td>-1.1 ± 3.9</td>
<td>&gt;0.7</td>
</tr>
<tr>
<td>Frequency (impulses/sec)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mm Hg</td>
<td>-12.4 ± 2.4</td>
<td>&lt;0.01</td>
<td>-25.3 ± 8.8</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>40 mm Hg</td>
<td>-7.0 ± 2.6</td>
<td>&lt;0.02</td>
<td>-14.8 ± 5.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>60 mm Hg</td>
<td>-4.2 ± 3.2</td>
<td>&gt;0.2</td>
<td>-20.4 ± 8.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>80 mm Hg</td>
<td></td>
<td></td>
<td>-18.8 ± 7.1</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

All values are means ± SE.
*Pressures given are pressures above original threshold pressure.

arch, and the descending thoracic aorta were also involved.

Typically there was extensive calcification of the inner aspects of the media with gross disruption of the normal architecture of the vessel wall. The elastic laminae were less wavy than those in normal rabbits and appeared fixed in an expanded position, and in many places they were ruptured and degenerated (Fig. 7). In other places the distance between the laminae appeared wider than that in normal rabbits, and there was an increase in the mucopolysaccharide content of the media demonstrated by the toluidene blue stain. In some rabbits the aortic arch contained cartilage, and in one rabbit the early formation of bone-containing erythroblastic-type cells occurred. There was intimal proliferation and fibrosis of the areas overlying the medial calcified lesions, and the internal elastic laminae were frequently degenerated.

Heart.—Sixteen of the 22 rabbits used in this series had pathological lesions involving the heart. There was calcification of the media of the arteries and arterioles with intimal proliferation and perivascular round cell infiltration. Coronary thrombosis was observed in one rabbit. The cardiac muscle was fibrosed or necrotic in places with increased cellularity. Nine rabbits had calcified lesions of the cardiac muscle, and cartilage was found in one heart. Four rabbits that died had evidence of recent cardiac infarction.

Kidney.—Nineteen of the rabbits that were fed the calciferol diet developed pathological lesions of the kidneys. There was calcification of the parenchyma involving the tubules, the glomeruli, and the arterioles. In many instances there was proliferation of the media and the intima of the arterioles, and the glomeruli showed signs of ischemia with atrophy and fibrosis. The rabbits with the most extensive calcification and glomerular damage died before the terminal experiment.

Baroreceptor Nerve Endings.—Normal baroreceptor nerve endings (4, 11) were stained in sections from the aorta and carotid sinus region of five of the calciferol-treated rabbits. In three rabbits some degenerated nerve endings similar to...
two rabbits with the lowest mean arterial blood pressure (rabbits 4 and 5) had minimal lesions affecting the aortic arch. Two other rabbits (nos. 3 and 10) with only minimal elevations in blood pressure had fairly extensive lesions with calcification, but they had previously had high mean arterial blood pressures. Two more rabbits with minimal lesions had mild hypertension; the level of blood pressure reached a plateau. The rabbit (no. 6) with the highest mean arterial blood pressure (175 mm Hg) had extensive aortic lesions, but the terminal experiment was carried out at an earlier stage than the experiments with the other rabbits; therefore, its blood pressure was probably at its peak value (Table 2). Two other rabbits (nos. 8 and 11) had extensive calcification of both the ascending aorta and the descending aorta, and both had elevated mean arterial blood pressures at the time of the terminal experiment of 143 mm Hg and 145 mm Hg, respectively.

**Discussion**

Rabbits given large doses of calciferol and calcium lactate developed medial sclerosis of the arteries, as first described by Kreitmair and Moll (12) and confirmed by others (13, 14). Although the treatment was continued for only a short period of time, progressive lesions occurred after the withdrawal of the calciferol even though the serum calcium level was normal. In rats too, serum calcium only rises during the first few days of treatment (14). These treated rabbits and rats thus differ from humans with hypervitaminosis D who have a high serum calcium level (15). This difference in the serum calcium levels may be related to the extent of the calciferol intoxication induced in the rabbits; many rabbits died as a result of the large doses of calciferol initially administered in this series. Furthermore, the progressive nature of the lesions may well have been the result of renal damage with the subsequent retention of phosphate, as demonstrated by the elevated serum phosphate level.

An elevation of serum cholesterol has been observed in humans with hypervitaminosis D (16) and was also apparent in the calciferol-treated rabbits only 10 days after the treatment was begun. To what extent this elevation in serum cholesterol is responsible for the subsequent intimal proliferation overlying the calcified medial lesions is difficult to ascertain, especially in the presence of hypertension (4, 17).

These complicated lesions produced by vitamin...
D in experimental rabbits are thought by some authors (14) to be more typical of human arteriosclerosis than are those produced by high cholesterol diets (15); however others (18, 19) consider them to be more typical of Mönckeberg’s sclerosis. Although some of the isolated lesions of the arteries may resemble both of these conditions, the response of the body as a whole is specific to hypervitaminosis D.

**BARORECEPTOR ACTIVITY**

Baroreceptor activity is directly related to the mechanical properties of the vessel wall in which they are situated (4, 7, 20). The baroreceptors have intimate connections with the elastic tissue and the smooth muscle of the adventitia and the media (4, 21, 22); therefore, they are affected by any alteration in the structure of the wall which, in its turn, affects the viscoelastic properties.

The gross, patchy nature of the lesions affecting the arteries of the calciferol-treated rabbits would thus give rise to nonuniformity of the viscoelastic properties of the different parts of the vessel wall and would explain the large range of functional activity of the individual baroreceptor fibers in any one rabbit in the group. The very irregular discharge pattern exhibited by some fibers when the pressure was elevated in steps, which resulted in a charge pattern exhibited by some fibers when the tension acting on the baroreceptor nerve endings would continue to stimulate the receptors even at low aortic pressures. As the aortic pressure is raised these same lesions would hinder expansion of the arterial wall at lower pressures than normal, and this phenomenon would account for the lower point of inflection in a majority of the baroreceptors. Receptors with higher than normal inflection points were presumably situated in a part of the vessel wall that was not splinted by calcium.

The shifting of the baroreceptor impulse frequency-pressure curves to the left in the majority of the calciferol-treated rabbits contrasts directly with the results obtained in similar experiments on rabbits with experimental renal hypertension (4) and with atherosclerosis (5, 20), in which the curves were shifted to the right of normal curves. The explanation for these differences almost certainly lies in the nature of the pathological lesions present in the three groups. Hence, the two calciferol-treated rabbits (nos. 4 and 5) that had no gross calcified lesions in the aortic arch had baroreceptors exhibiting physiological characteristics comparable with the changes that occur in renal hypertension (4).

The degree of shift of the curves and the reduction in the baroreceptor sensitivity were related to the time elapsing from the commencement of the calciferol treatment and, hence, to the length of time the rabbits had been hypertensive, although there was no direct correlation between the shift of the impulse frequency-pressure curves and the mean arterial blood pressure of these rabbits at the time of the terminal experiment. As in other studies (4, 20), there was some correlation between the reduction in the baroreceptor sensitivity and the elevation in the mean arterial blood pressure which was higher in those rabbits that had the most extensive lesions of the aorta. This reduction in the baroreceptor activity was associated with a reduced distensibility of the arterial walls.

The degree of the reduction in the distensibility was related to the extent of the pathological process involving the vessel walls, particularly to the amount of calcification, and was more marked in the present group of calciferol-treated rabbits than it was in rabbits with experimental renal hypertension (4) or experimental atherosclerosis (20), especially at the higher pressures. The decreased distensibility was probably enhanced by the changes in water and electrolyte content of the wall resulting either from the hypertension per se or from the involvement of the kidney in the pathological processes.

The relationship between the changes in mechanical properties of the aortic arch and baroreceptor sensitivity is further emphasized by comparison of three groups of rabbits having experimental arteriosclerosis with normal rabbits (23, 24). In the arteriosclerotic rabbits there is an inverse relationship between the distensibility of the aorta and the sensitivity of the baroreceptors. All three pathological groups had aortas less distensible than those of normal rabbits with less sensitive baroreceptors. In comparing the three pathological groups, it was evident that, although the calciferol-treated rabbits had the least distensible aortas and the least sensitive baroreceptors, the atherosclerotic rabbits had the most distensible aortas and the most sensitive baroreceptors. The
renal hypertensive rabbits came between the other two groups both with regard to vessel distensibility and sensitivity although as a group they had the highest mean arterial blood pressure (23, 24).

The hysteresis response, which occurs during stepwise increases and decreases in aortic pressure (7), was also more exaggerated in the calciferol-treated rabbits than it was in any of the other groups previously studied (4, 7, 20); hence, it too appeared to be directly related to the amount of the reduction in the elasticity of the vessel walls.

BARORECEPTOR ACTIVITY AND BLOOD PRESSURE

All of the calciferol-treated rabbits had mild-to-severe hypertension. The diastolic pressure was elevated, and the systolic pressure was elevated even more; on the average the pulse pressure was about twice the normal value. These changes were consistent with an increased peripheral vascular resistance and with reduced distensibility of the aorta.

A reduction in the distensibility of the arterial wall has previously been implicated in the etiology of hypertension of experimental atherosclerosis (5, 20) and renal hypertension (4, 25) by virtue of its effect of reducing baroreceptor activity and sensitivity leading to a reflex increase in peripheral vascular resistance. Splinting the carotid sinuses with a plaster cast also causes hypertension (26). A similar mechanism probably plays an important part in the hypertension observed in the calciferol-treated rabbits and may contribute to the hypertension that occurs in patients with hypervitaminosis D (6) and Takayashu’s disease; these conditions are characterized by extensive calcified lesions involving the arteries and the regions of the arteries in which the baroreceptors are situated.

Although no measurements of cardiac output were made, the rise in blood pressure almost certainly resulted from an increase in peripheral vascular resistance, since the cardiac output, at least in humans with atheroma, falls in many cases probably as a result of coronary atheroma, and from cardiac infarction. Lesions affecting the coronary vessels and cardiac muscle were also observed in the calciferol-treated rabbits.

An increase in peripheral vascular resistance could be the result of several mechanisms acting singly or in combination: a narrowing of the blood vessels due to pathological changes, a humoral factor from the kidneys due to renal vessels and renal parenchyma being involved in the process of medial sclerosis and calcification, and an increase in sympathetic adrenergic nerve activity. The latter could be brought about partly by the reduced baroreceptor activity at higher aortic pressures described in this paper, although there is less inhibition of the “vasomoter center.” However at the lower range of pressures baroreceptor activity will be greater than normal, as demonstrated in whole-nerve recordings (unpublished observations), which would result reflexly in less than normal sympathetic activity. The observation that the arterial blood pressure of the calciferol-treated rabbits was extremely labile could be explained by these modifications of baroreceptor activity combined with the diminished recruitment of other baroreceptors since so many of the receptors have very low threshold pressures. There may be some loss of receptor activity due to baroreceptor degeneration which would contribute to these effects. The lability of the blood pressure was greater in the calciferol-treated group than it was in either the renal hypertensive group or the atherosclerotic groups and, hence, it was directly related to the reduction in baroreceptor sensitivity and the reduced distensibility of the aortic arch (23, 24).

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