Studies on the Pathogenesis of Hypertensive Vascular Disease

EFFECT OF HIGH-PRESSURE INTRA-ARTERIAL INJECTIONS IN RATS
By Gary S. Hill, M.D.

ABSTRACT
Repeated high-pressure injection of saline into the arterial system via carotid cannula in normal, ether-anesthetized rats has been previously shown to produce acute necrotizing lesions in the renal vasculature. In this study administration of the vasodilator aminophylline prior to high-pressure injections led to similar lesions, produced in dilated, hyperdistended vessels, at far lower pressures than in control animals, demonstrating that neither vasoconstriction nor the absolute height of blood pressure is a critical determinant in production of lesions. In the second part of the study previous treatment with desoxycorticosterone (DOCA)-saline for 12 to 18 days rendered the renal vasculature abnormally prone to lesions after high-pressure injections, compared with normal rats so injected or those receiving a DOCA-saline regimen only. Glomerular lesions were more severe than can be explained on the basis of simple addition of the lesions resulting from high-pressure injections to those arising from DOCA administration. This increased susceptibility of the vasculature to elevated pressure is probably a function of focal alteration of vascular smooth muscle. These observations support the proposal that hypertensive vascular lesions occur in dilated, hyperdistended vessels and the glomeruli distal to them, the dilatation probably resulting from focal smooth muscle alteration with partial or complete loss of contractility.

ADDITIONAL KEY WORDS: glomerulus, smooth muscle, vasodilator, kidney, aminophylline, saline injection, DOCA, hypertension

For many years, opinion influenced greatly by Volhard (1) held that the vascular lesions of hypertension arise in constricted arteries and arterioles as the result of focal ischemia and necrosis of the wall. However, in a long series of elegant observations in hypertensive rats Byrom (2-5) has described an arterial pattern consisting of segments of arteries showing intense vasoconstriction alternating with overdistended dilated segments, the artery thus resembling a string of sausages. Others (5-7) have observed this pattern in various models of acute and chronic hypertension. These observers (5-7) feel that vascular lesions actually occur in the focally dilated vascular segments. Byrom (5) believes that at the highest levels of pressure the weaker segments of resistance vessels give way, plasma appears in the vessel wall, and necrosis of the smooth muscle occurs, suggesting that the essential lesion is a local overstretching or tearing of muscle fibers. He feels that necrosis is particularly likely to occur in vessels that have been weakened in any way, whether by physical damage or atrophy and dilatation.

Our own observations (6) from an earlier microangiographic study of steroid hypertension in the rat supported the notion that vascular lesions in that condition arise in dilated, rather than constricted, segments. In addition, they suggested that steroid administration may have the double effect not only of creating hypertension, but of rendering the vasculature more than normally susceptible to the effects of the increased pressure. This
increased susceptibility appears to be due to focal alteration of vascular smooth muscle cells, commencing even before onset of hypertension and resulting in partial or complete loss of contractility.

In this study, these questions were examined with the technique of high-pressure intraarterial saline injections developed by Byrom and Dodson (8). They showed that sudden increase of intravascular pressure itself may produce necrotizing vascular lesions in the kidneys of normal rats. One portion of this study sought to strengthen the initial proposition that vascular lesions occur in dilated rather than constricted vessels by examining the effects of high-pressure injection in rats receiving large doses of the vasodilator aminophylline (theophylline ethylenediamine), to rule out any possible role of vasospasm in production of lesions. In the other portion of the study, the high-pressure injection technique was used to demonstrate that following steroid administration there is indeed increased vulnerability to the effects of elevated pressure.

Materials and Methods

Female Holtzman rats weighing 200 to 225 g were used. All studies were carried out with the animals under open ether anesthesia. In the first part of this study normal rats were used. These were divided into two groups: (1) aminophylline-injection, these received 40 mg aminophylline intravenously followed immediately by a series of high-pressure saline injections (see below), (2) aminophylline-control rats, these received injection of 40 mg aminophylline only, without succeeding saline injections. Each experimental group was in turn divided into two subgroups, one used to measure the blood pressure changes induced by the various experimental procedures (see below) and killed after measurements, and the second was used for histologic study. These rats died or were killed at intervals varying from 6 hours to 3 days after operation. Aminophylline-injection rats tended to die at periods of 6 to 24 hours. This was not true of either the control-injection (see below) or aminophylline-control groups; save for modest weight loss, these groups tolerated the procedures quite well and appeared healthy at the time they were killed.

The rats used in the second half of the study initially had a left nephrectomy followed by 12 to 14 days of recovery. They were divided into four experimental groups. All groups received twice-weekly injections of 0.5 ml of a steroid preparation containing in each milliliter 25 mg desoxycorticosterone trimethylacetate (DOCA) in a medium containing 10.5 mg methylcellulose, 3 mg sodium carboxymethylcellulose, and 1 mg polysorbate 80, all to facilitate suspension. They were given a solution of 0.5% NaCl and 0.49% KCl to drink. Two groups, DOCA-injection rats, received high-pressure intra-arterial saline injections (see below), one group on the twelfth day and the other on the eighteenth day of the steroid-saline regimen. The first were killed 3 days later, on the fifteenth and twenty-first day, respectively. The second two groups, DOCA-control rats, served as controls for the first two groups and were also killed on the fifteenth and twenty-first day. They were maintained on the same steroid-saline regimen but did not have high-pressure injections.

A control group of normal rats referred to as control-injection rats was also submitted to high-pressure saline injection. This group was also divided into two subgroups, one used to measure blood pressure changes during high-pressure injections (see below) and sacrificed immediately afterward, the other killed 3 days after injections and the kidneys studied histologically. The technique of repeated high-pressure intraarterial saline injection was similar to that originated by Byrom and Dodson (8). Under ether anesthesia, the carotid artery was cannulated with a 19-gauge needle with smoothed bevel. This needle was connected by a short, 10-inch thick-walled polyethylene tubing to a 10-ml Luer type of syringe. Isotonic saline with 5.0 mEq/liter KCl and 2.0 units/ml heparin was used for injection. The most satisfactory technique of injection was to steady the barrel of the syringe with one hand while giving a sudden burst of pressure against the plunger with the opposite palm and instantly releasing the pressure. The amount injected was usually between 1 and 2 ml. After each injection, an amount roughly equivalent to that injected was allowed to run back from the carotid into the tubing. Thus a maximum number of injections could be made with a minimal total volume of saline; as many as 20 injections could be made with a total amount of approximately 5 to 7 ml injected. The interval between injections was generally 30 to 60 seconds.

The blood pressure changes generated by saline injections and by aminophylline administration were measured directly in animals from the following groups: control-injection, aminophylline-injection, and aminophylline-control. Here the abdominal aorta was cannulated just above the iliac bifurcation with an 18-gauge needle con-
HYPERTENSIVE VASCULAR DISEASE

Typical tracing of the blood pressure changes during a high-pressure saline injection in a normal rat.

Results

BLOOD PRESSURE ALTERATIONS

As Wölfgarten and Magarey (1) found with this injection technique, the blood pressure rises almost instantaneously to very high levels and falls equally abruptly to near baseline levels, in a span of 1 to 2 seconds (Fig. 1). The maximum systolic pressure achieved on saline injection in control animals was 308 mm Hg (Table 2), and the average for all injections was 239 mm, with pressures of 250 mm or greater being obtained in all animals on at least one injection. The average rise above baseline levels was 133 mm; the maximum rise was 200 mm Hg.

Aminophylline altered these responses very considerably (Table 2). Injection of 40 mg aminophylline lowered the blood pressure to the range of 50 to 70 mm systolic and 30 to 40 mm diastolic. Pressure response to high-pressure injections was also reduced, not only in terms of the maximum levels reached but also in the amount of rise. The maximum pressure reached in any animal was 228 mm, with the maximum pressure in each animal averaging 165 mm; the average peak pressure for all injections was 138 mm. Rises in pressure were similarly reduced, the average rise being only 81 mm Hg.

ARTERIAL AND ARTERIOLAR LESIONS

Lesions took two basic forms. The first consisted of hyaline material staining similarly to plasma, deposited in varying amounts in the intima, under the internal elastic lamina, or between and around the intact smooth
Preinjection Systolic Blood Pressure (mm Hg) Alterations in Groups Used to Study Effects of Steroid Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Preinjection</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-day DOCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOCA-injection</td>
<td>(6)</td>
<td>118 ± 11</td>
<td>134 ± 12</td>
</tr>
<tr>
<td>DOCA-control</td>
<td>(6)</td>
<td>118 ± 6</td>
<td>149 ± 19</td>
</tr>
<tr>
<td>21-day DOCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOCA-injection</td>
<td>(15)</td>
<td>115 ± 7</td>
<td>137 ± 11</td>
</tr>
<tr>
<td>DOCA-control</td>
<td>(5)</td>
<td>123 ± 6</td>
<td>137 ± 10</td>
</tr>
<tr>
<td>Saline injection only</td>
<td>(13)</td>
<td>118 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

Number of rats is in parentheses.

Response of Systolic Blood Pressure (mm Hg) to High-Pressure Intrarterial Saline Injection

<table>
<thead>
<tr>
<th>Number</th>
<th>Maximum peak pressure</th>
<th>Average peak pressure</th>
<th>Maximum rise</th>
<th>Average rise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control-Injection Rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>392</td>
<td>250</td>
<td>243</td>
<td>100</td>
<td>121</td>
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<tr>
<td>383</td>
<td>260</td>
<td>259</td>
<td>190</td>
<td>152</td>
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<td>384</td>
<td>305</td>
<td>296</td>
<td>176</td>
<td>148</td>
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<td>385</td>
<td>295</td>
<td>286</td>
<td>172</td>
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<td>386</td>
<td>280</td>
<td>270</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>387</td>
<td>277</td>
<td>261</td>
<td>194</td>
<td>139</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>277 ± 53</td>
<td>230 ± 28</td>
<td>176 ± 21</td>
<td>133 ± 18</td>
</tr>
</tbody>
</table>

|        | Aminophylline-Injection Rates|                       |              |              |
| 416    | 152                   | 128                   | 130          | 75           |
| 417    | 150                   | 124                   | 119          | 66           |
| 418    | 130                   | 121                   | 73           | 59           |
| 419    | 164                   | 141                   | 122          | 84           |
| 420    | 545                   | 176                   | 176          | 165          |
| Mean ± SD | 185 ± 37 | 136 ± 19 | 150 ± 37 | 81 ± 10 |

*Above baseline systolic pressures.
Fibrinoid necrosis of an interlobular artery in an animal receiving desoxycorticosterone trimethylacetate (DOCA) for 18 days then high-pressure saline injections and killed 3 days after injections. PAS. Line = 50 μ.

were quite extensive at all levels of the arterial tree.

**GLOMERULAR LESIONS**

The present results are similar to those of Byrom and Dodson (8) and of Wolfsgarten and Magarey (10) in that it was quite impossible to cause any glomerular fibrinoid necroses in normal animals receiving saline injections only. It was possible, however, to produce infrequent glomerular fibrinoid necroses in some of the DOCA-injection rats, although none were seen in DOCA-control rats.

Far more common were deposits of eosinophilic, PAS-positive material in the glomerular tuft (Fig. 3), often seen in relation to the capillary basement membrane or lying in the concavity of the capillary loops. It was also frequently found in the mesangium where it often took the form of small hyaline droplets. These lesions, identical in form and varying only in severity, were present in these experimental groups: DOCA-injection, DOCA-control, aminophylline-injection, and control-injection. Only in the aminophylline-control group were they not present to any significant extent. In rats receiving DOCA, these lesions were associated with the clear vascular glomerular lesions resulting from accumulations of the methylcellulose in the suspension medium of the steroid preparation, deposited primarily in mesangial cells, as previously described (11, 12).

Also, many of the aminophylline-injection rats and one DOCA-injection rat had numerous glomeruli containing hyaline or fibrinous capillary thrombi (Fig. 4). These capillary thrombi, described in other studies on experimental hypertension (13, 14), were not seen in any of the control groups.

To compare the different experimental groups, an attempt was made to quantify the extent and severity of glomerular lesions. A glomerular lesion index (GLI), similar in concept to the juxtaglomerular index (JGI) (15), was calculated for each kidney as follows:

Lesions were graded 0 to 4+ (Fig. 5) on the basis of the extent of the glomerulus showing evidence of involvement:
FIGURE 3
Glomerulus from a normal rat receiving high-pressure saline injections, showing numerous hyaline droplets in the substance of the tuft, together with infrequent areas of capillary basement membrane thickening. PAS. Line = 40 μ.

FIGURE 4
Glomerulus from rat receiving DOCA for 12 days then saline injections. Note the abundant capillary thrombi of fibrin-like material and the clearly fibrillar structure of these thrombi when seen in longitudinal section. This is from the sole DOCA-injection animal which developed such fibrin thrombi, although they were present in numerous ammonium-injection animals. By contrast, no control-injection animal showed such lesions. PAS.
Examples of the approximate severity of glomerular lesions classified as Grade 1 (A), Grade 2 (B), Grade 3 (C), and Grade 4 (D). Observe that the severity of lesions tends to increase out of proportion to the extent of the glomerulus involved. PAS; calibration equals 50 μ.

0 = No evidence of lesions;
1+ = Up to 1/4 of tuft showing evidence of involvement;
2+ = 1/2 of tuft involved;
3+ = 3/4 of tuft involved;
4+ = Most to all of tuft involved.

Excluded from consideration were the methylcellulose vacuolar lesions in rats receiving DOCA. These were of equal severity in saline-injected and control-DOCA rats and were probably not pressure-related, for they may appear before onset of hypertension (11). From each kidney, 100 glomeruli, taken from all levels of the cortex, were graded. The totals recorded under one-, two-, three-, and four-plus were multiplied by the factors 1, 2, 4, and 8, respectively, for purposes of quantification, because it was noted that with increasing involvement of the glomerular tuft the severity of lesions increased quite out of proportion to the extent of involvement (so that, in a sense, three 1+ lesions added together would not have equaled a 3+ lesion in severity). The subtotals for each grade were added to give a cumulative total or glomerular lesion index.

In the portion of the study dealing with the effects of DOCA administration, the severity of lesions was much greater in the DOCA-injection groups at both 15 and 21 days than...
Comparison of the glomerular lesion index values of the control-injection animals with the 15-day experimental group (A) or the 21-day experimental groups (B). Mean and SD are indicated in each instance for the control-injection and DOCA-control groups. Broken lines in the columns for the DOCA-injection groups represent the sums of the means and SD of the first two groups in each instance (see text). Note that a large proportion of the animals in both the 15-day and 21-day DOCA-injection groups fall above these levels, indicating that the lesions in these animals are more severe than might be expected from simple addition of the lesions resulting from saline injection to those caused by DOCA administration. This suggests that DOCA administration in some manner "sensitizes" the vasculature to the effects of increased pressure.

in either the DOCA-control or control-injection groups (Fig. 6), as had been anticipated. However, one possible explanation for this increased severity might be that because baseline pressures were elevated in these rats (many of which were mildly hypertensive at the time of saline injection) the maximum pressures attained on injection were higher, and hence the lesions worse. However, as seen from Figure 7, there is no relationship between the baseline blood pressure and the severity of lesions attained in DOCA-injection rats. Another explanation might be that the increased severity of lesions in the DOCA-injection group was simply the additive result of combining saline injection and DOCA administration in the same animal. To support the proposal that DOCA administration sensitizes the vasculature to the effects of increased pressure, it was necessary to demonstrate that the severity of lesions in the DOCA-injection groups was greater than might be anticipated from superimposition of the lesions from one procedure upon those resulting from the other. The probability that the GLI of a given animal in response to either procedure alone will be greater than 1 SD above the mean for that procedure, i.e., $x + 1\sigma$, is approximately 15.86%. If one presumed that the lesions from these two procedures were simply additive, then, even if an animal’s response to the two procedures
totally paralleled one another, certainly no more than 15% of animals would be expected to have GLI values greater than the sum $\sum (x + \sigma)$ of the means + $\sigma$ of the DOCA-control and control-injection groups. Similarly, no more than about 2.2% would be expected to fall above the sum of the means plus 2 $\sigma$, $\Sigma (x + 2\sigma)$.

However, as seen in Figure 6, 7 of 15, or 46%, of the 21-day DOCA-injection rats and 3 of 6, or 50%, of the 15-day DOCA-injection rats fell above the $\sum (x + 1\sigma)$ level as, more importantly, did the means of both groups. Quite a substantial number of GLIs fell above the $\Sigma (x + 2\sigma)$ level. Furthermore, most values were sufficiently higher than the $\sum (x + 1\sigma)$ level to make it seem improbable that the differences can be accounted for by lack of precision in grading the severity of lesions. Thus it appears highly likely that the steroid-saline regimen does indeed alter the blood vessels in some manner, predisposing them to development of lesions on increased pressure.

A similar approach was used in the study of the effects of aminophylline administration. A GLI was calculated for each kidney (Fig. 8). Saline injection produced a moderate degree of glomerular damage in control rats (GLI = 40.3 ± 20.4) and considerably more severe lesions in those also receiving aminophylline (GLI = 117 ± 90.2). Rats receiving aminophylline only showed a low GLI value (10.8 ± 4.0). These low values could be due in part to a background level of false-positive identifications of cutting and staining artifacts confused for lesions and in part to subjective error in evaluating presence or absence of minimal lesions in these slides, which were randomized and graded blindly. Nonetheless, for statistical purposes, the most conservative assumption is that they are indeed the result of aminophylline administration. It is evident from inspection that the severity of lesions in the aminophylline-injection group was greater than anticipated from simple addition of the lesions resulting from the combination of aminophylline administration and saline injection. And, following the approach and reasoning discussed above, it is seen in Figure 8 that 6 out of 10, or 60%, of the aminophylline-injection group had GLIs above the $\sum (x + \sigma)$ level of 75.5 compared to the 15% expected if the lesions were merely additive. Furthermore, the mean GLI for the group (117.0)
was substantially higher than the $\Sigma (x + \sigma)$ level.

Discussion

In this study on the pathogenesis of hypertensive vascular lesions, the technique of high-pressure saline injections was chosen to create an acute mechanical hyperdistention of the vessels as the damaging force. It might be argued that the saline, rather than elevated pressure, was the noxious agent producing lesions. However, against this is the fact that, as other investigators have found (16, 17), unless injections are given at sufficient pressure, normal rats may be given rather massive injections of saline without developing any lesions whatever. This points out clearly that elevated pressure is necessary for production of lesions in normal animals. The possibility cannot be ruled out that saline would have itself caused modest lesions in the DOCA-rats with their tendency to sodium retention, but it seems unlikely that they would have been of any great magnitude. (Control experiments using sodium-free solutions could be performed, but any such solution in the volumes used in this experiment would inevitably lead to significant electrolyte shifts which might also be incriminated in the development of lesions.)

To study the proposition that vascular lesions occur primarily in dilated rather than constricted vessels, we induced maximal vasodilatation with aminophylline, paralyzing the vasculature with rather massive doses to minimize changes in active tension in response to pressure changes. This ruled out any possible vasoconstriction or reactive "spasm" in the production of lesions on high-pressure injections. This had not been possible in earlier studies producing vascular lesions with conventional vasoconstrictors, or in earlier saline-injection studies in which there existed the possibility of vascular spasm in reaction to the pressure bursts.

We successfully produced lesions in aminophylline-treated rats (Fig. 8), and these were indeed much more severe than those produced in normal animals. This demonstrates that morphologically typical "hypertensive" lesions can be produced in dilated vessels in the absence of any vasoconstriction whatever. In fact, in several rats multiple glomerular fibrin thrombi were observed in addition to the typical eosinophilic lesions. These were not found in any of the control-injection animals and in only one DOCA-injection rat. It appears that high-pressure injections here, because the vascular bed was so widely dilated, actually mechanically disrupted the endothelial cells with release of clotting factors and consequent intravascular coagulation. If this is so, it attests to the relatively greater forces brought to bear on the capillary endothelium by high-pressure injection in a dilated vascular bed, despite the fact that neither the degree of rise nor the absolute pressure obtained is as high as in the normal rat receiving saline injections. This also emphasizes that the absolute height of the pressure is not the critical determinant in development of lesions.

In an earlier microangiographic study of DOCA hypertension in rats (8) we observed that even before the onset of hypertension there appeared to be focal areas of relative vascular dilatation. It was suggested that in these areas there was functional alteration of the smooth muscle, with weakening and increased susceptibility to formation of vascular lesions as hypertension developed. The present study, as seen from Figure 6, confirmed this increased susceptibility and demonstrated that much more severe lesions can be produced by high-pressure injections in DOCA-treated rats than in normal rats. This study provides no direct information about the nature of this increased susceptibility to vascular lesions. We feel it is probably best interpreted as representing functional damage to the vascular wall without recognizable morphological damage (at least at the light microscopic level) and is manifested by the focal vasodilatation seen microangiographically. One can then view elevated pressure not as producing vascular lesions but, in an almost photographic sense, as "developing" them by causing deposition of a morphologically rec-
ognizable residue at the sites of functional damage where overdistention of the vessel wall and increased permeability occur.

Other studies have indicated increased susceptibility to vascular lesions in steroid-hypertensive rats, in the so-called "steroid-renin" syndromes where steroid administration, be it DOCA (18, 19), cortisone (14), hydrocortisone (20) or aldosterone (21), "conditions" for development of severe necrotizing vascular lesions when renin or angiotensin is given in doses that produce only minimal lesions in control animals. Various synergistic actions of renin or angiotensin with the steroids have been suggested to explain this phenomenon. However, a simpler explanation, particularly in light of the present studies, is that renin and angiotensin are acting primarily as simple pressor agents in these syndromes. These earlier studies and the present one are, of course, quite analogous. They emphasize that the basic alteration in these situations is focal weakening and increased vulnerability to development of lesions when the pressure is raised, whether by mechanical or pharmacologic means.

Byrom (5) feels that vessels weakened in any way show increased susceptibility to hypertensive damage. He cites the work of Asscher et al. (22), who observed that X-irradiation of mesenteric vessels so alters them that severe necrotizing lesions develop on induction of a modest degree of hypertension apparently on the basis of physical damage to the elastic lamina and other components of the vessel wall. Also cited are Byrom's own studies (5) showing that in rats with a Goldblatt type of hypertension, large doses of angiotensin cause lesions in a paradoxical distribution. Vessels in the clipped kidney show severe necrotizing lesions, whereas lesions in the undipped kidney are minimal. The explanation given is that the arterioles in the clipped kidney are dilated and involved because of the relatively low pressure beyond the clip, and are therefore especially vulnerable to necrosis when the angiotensin causes high pressures even distal to the clip. By contrast, arterioles in the opposite kidney, having undergone extensive compensatory medial hypertrophy, are relatively immune to development of lesions despite exposure to even higher pressures than those in the clipped kidney.

Although the above examples demonstrate the significance of weakening of the vessel wall in predisposing to lesions, the present studies offer the first suggestion that vascular weakening may not be limited to isolated special situations but may develop pari passu with increasing pressure in some models of experimental hypertension. The animal given steroids is thus doubly jeopardized, not only in becoming hypertensive but in having less than normal defenses against its effects. It will be of interest to investigate other models of experimental hypertension to see if such increased vascular susceptibility exists elsewhere. If so, this may help to explain why different experimental models differ so greatly in their proclivity to produce lesions.

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