Effects of Two Forms of Hypertension on Atherosclerosis in the Hyperlipidemic Baboon

Henry C. McGill, Jr., Kenneth D. Carey, C. Alex McMahan, Yolan N. Marinez, Thomas E. Cooper, Glen E. Mott, and Colin J. Schwartz

We examined the relationship of hypertension and plasma renin activity to atherogenesis in 48 moderately hyperlipidemic (total serum cholesterol was about 200 mg/dl) baboons (Papio sp.). We used renal artery stenosis (two-kidney, one clip model) to produce hypertension associated with elevated plasma renin activity, and used cellophane wrapping of both kidneys (bilateral perinephritis model) to produce hypertension with normal renin activity. Renal artery stenosis and bilateral perinephritis increased both systolic and diastolic blood pressure by about 30 mm Hg. Renal artery stenosis approximately doubled, but bilateral perinephritis did not change plasma renin activity.

Both hypertensive groups, to about the same degree, had significantly more extensive atherosclerosis than the control group in the abdominal aorta and brachial, iliac-femoral, and carotid arteries. The effect of hypertension was greatest in the carotid arteries where the extent of atherosclerosis was nearly tripled. Hypertension did not influence lesions in the thoracic aorta.

By multiple regression analysis, very low plus low density lipoprotein cholesterol, high density lipoprotein cholesterol, and systolic blood pressure were consistently strong predictive variables for the extent of atherosclerotic lesions. Most of the effects of renal hypertension on atherosclerotic lesions appeared to be accounted for by the increase in blood pressure. In the carotid arteries, however, there was a suggestion of an effect above that due to increased blood pressure. Additional analyses indicated that these treatment effects were associated with serum potassium concentration, plasma renin activity, or other closely related variables.

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Hypertension has long been known to increase the frequency of coronary heart disease and stroke, and also to increase the extent and severity of atherosclerosis in both humans and experimental animals. However, the mechanism of this effect remains uncertain. Some evidence from hypertensive humans indicates that high plasma renin activity (PRA) is associated with increased risk of coronary heart disease and stroke. The knowledge of whether plasma renin accelerates atherogenesis is important because some antihypertensive drugs elevate PRA. The failure of antihypertensive therapy to reduce the risk of coronary heart disease as much as it reduces the risk of stroke and heart failure could be due, in part, to the effects of drug therapy on renin secretion.

To address this issue, we compared the effects of two types of experimental hypertension, one associated with normal, and the other with elevated, PRA on atherogenesis in baboons with moderate diet-induced hyperlipidemia.

Methods

Subjects

The subjects were 59 feral adult male baboons (Papio cynocephalus) purchased from importers. The animals were fed a commercially prepared baboon chow (Ralston Purina Company, St. Louis, Missouri); tested for tuberculosis; and treated for parasites during a 5-week quarantine period. Their...
weights averaged 26.0 kg (range, 19.2 to 35.5 kg), and their estimated ages averaged 9 years (range, 5 to 16 years). Initially, the baboons were housed in outdoor gang cages, but they were transferred to indoor, individual cages at the beginning of the preliminary challenge period. They were kept on a 12-hour light/dark cycle (6 AM to 6 PM) and were fed once daily. We divided them into four blocks for management purposes and kept them within these blocks throughout the experiment.

Eleven animals died during the 9 months of preliminary dietary challenge or the 13 months of experimental hypertension. These animals were excluded from the statistical analyses, which are based on the 48 animals that completed the experiment.

The protocol for this experiment was approved by the Animal Research Committee of the Southwest Foundation for Biomedical Research.

Blood Collection

When scheduled, we collected arterial blood in vacutainers from each baboon after an overnight fast and under ketamine immobilization (10 mg/kg of Vetalar, Parke, Davis & Company, Detroit, Michigan).

Cholesterol and Lipoprotein Analyses

We measured cholesterol in whole serum and in the supernatant after dextran sulfate-CaCl₂ or heparin-manganese precipitation by an enzymatic method using the ABA 100 Bichromatic Analyzer (Abbott Laboratories, South Pasadena, California). The dextran sulfate-CaCl₂ procedure was used only for samples taken during the preliminary dietary challenge, and the heparin-manganese procedure was used for all analyses during the definitive experiment. Very low density lipoprotein cholesterol plus low density lipoprotein cholesterol (VLDL-C + LDL-C) were calculated as the difference between total serum cholesterol and high density lipoprotein cholesterol (HDL-C). The heparin-manganese procedure met the criteria of the Lipid Standardization Program of the Center for Disease Control. The coefficient of variation for duplicate analyses was less than 2%.

Plasma Renin Activity

We used radioimmunoassay of angiotensin I generated in incubated plasma samples to determine PRA. The Ⅱangiotensin, antibodies, and standards were obtained in kits (E.R. Squibb and Sons, Incorporated, Princeton, New Jersey). We calculated the results (ng/ml/hr) by the log-logit program of Rodbard.

Electrolytes and Other Blood Chemical Analyses

We measured electrolytes using a flame photometer with an internal lithium standard. Other blood chemistry analyses were performed by National Health Laboratories, Incorporated, San Antonio, Texas, by using a Technicon Corporation, SMAC-1 20-channel multichromatography analyzer.

Dietary Hyperlipidemia

While the animals were still on baboon chow after release from quarantine, we drew blood samples three times at 4-week intervals. We then fed the animals a diet enriched in cholesterol and saturated fat (Table 1) for 36 weeks and drew blood samples at 4, 16, 20, 24, 28, and 36 weeks. The serum cholesterol and lipoprotein cholesterol levels approximately doubled while the animals were on the enriched diet (Table 2). The animals were returned to a chow diet while the surgical procedures to induce hypertension were performed. We began to feed them the fat- and cholesterol-enriched diet again 2 months after the surgical procedures and continued this until the end of the experiment.

Preliminary Comparison of Experimental Hypertension Methods

To develop reliable procedures for inducing hypertension with high and low renin activity, we conducted preliminary experiments with four procedures, one of which (two-kidney, one clip) was associated with elevated PRA and three of which (one-kidney, one clip, bilateral perinephritis, and deoxycorticosterone-salt) were not associated with elevated PRA. We found that the two-kidney, one clip and the bilateral perinephritis models were the most reliable in producing moderate increases in systolic and diastolic blood pressure of 20 to 50 mm Hg. Consequently, we selected these two procedures to induce hypertension with contrasting levels of PRA.

Experimental Design

A previous experiment had shown that the VLDL-C + LDL-C/HDL-C ratio was a good predictor of experimental atherosclerosis. Therefore, within

<table>
<thead>
<tr>
<th>Table 1. Composition of Atherogenic Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
</tr>
<tr>
<td>Ralston Purina Special</td>
</tr>
<tr>
<td>Monkey Chow 25-5045-6</td>
</tr>
<tr>
<td>Lard</td>
</tr>
<tr>
<td>Coconut oil</td>
</tr>
<tr>
<td>Dried egg yolk</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Vitamin C</td>
</tr>
<tr>
<td>Retinol acetate</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

When mixed with water for pelleting, the diet provided the following: cholesterol, 4.0 mg/Kcal; sodium, 5.8 mEq/100 Kcal; potassium, 4.4 mEq/100 Kcal; energy, 377 Kcal/100 g.
Table 2. Total Serum and Lipoprotein Cholesterol Concentrations and VLDL + LDL/HDL Cholesterol Ratios of Experimental Groups before and during Preliminary Challenge

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Control</th>
<th>Renal artery stenosis</th>
<th>Bilateral perinephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>17</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Total serum cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>96.5 (17.4)</td>
<td>98.0 (11.0)</td>
<td>98.0 (13.1)</td>
</tr>
<tr>
<td>Challenge</td>
<td>198.3 (44.1)</td>
<td>199.3 (34.9)</td>
<td>208.1 (48.2)</td>
</tr>
<tr>
<td>VLDL + LDL cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>28.5 (8.6)</td>
<td>29.7 (7.8)</td>
<td>28.7 (7.6)</td>
</tr>
<tr>
<td>Challenge</td>
<td>85.1 (31.7)</td>
<td>85.1 (31.1)</td>
<td>89.2 (33.2)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>64.3 (11.3)</td>
<td>65.9 (8.6)</td>
<td>65.4 (10.4)</td>
</tr>
<tr>
<td>Challenge</td>
<td>109.4 (15.8)</td>
<td>109.3 (16.0)</td>
<td>112.3 (18.0)</td>
</tr>
<tr>
<td>VLDL-C + LDL-C/HDL-C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.44 (0.12)</td>
<td>0.45 (0.14)</td>
<td>0.44 (0.14)</td>
</tr>
<tr>
<td>Challenge</td>
<td>0.78 (0.33)</td>
<td>0.78 (0.30)</td>
<td>0.79 (0.28)</td>
</tr>
</tbody>
</table>

Values are means, and numbers in parentheses are standard deviations. Cholesterol concentrations were determined by the dextran sulfate-CaCl₂ precipitation method (see reference 20).

Procedure for Renal Artery Stenosis

For each surgical procedure, we immobilized the animal with an intramuscular injection of ketamine (10 mg/kg) in the biceps femoris. Inhalation anesthesia was administered with a mixture of halothane (1.5% vol/vol) and nitrous oxide (40% vol/vol) through an intratracheal cannula. We gauged the depth of anesthesia adequate for surgery when the palpebral reflex was abolished and when we could elicit no tension in the muscles of mastication upon extending the mandible. This depth of anesthesia was maintained until skin suturing was completed. A midline incision extending from the xiphoid process to the umbilicus provided access to both kidneys. We dissected the left renal artery free from the aorta to the renal hilus. A Goldblatt clip was loosely attached to the renal artery adjacent to the aorta, and a flow probe (Zapeda SWF-4 Electromagnetic Square Wave Flowmeter, Zapeda Instruments, Incorporated, Seattle, Washington) was attached to the artery adjacent to the kidney. After renal artery flow stabilized, we tightened the Goldblatt clip until flow was reduced by 50% and observed the flow for 15 minutes. If the flow deviated by more than 0.5 unit during this period, we released the clip and repeated the procedure until a stable reduced flow for 15 minutes was achieved.

Procedure for Bilateral Perinephritis

The anesthesia and incisions were identical to those used for renal artery stenosis. We dissected each kidney from its retroperitoneal space and stripped its capsule. A perforated, moistened, 25 x 36 cm cellophane strip (215PD cellophane, E.I. Du Pont de Nemours Company, Incorporated, Wilmington, Delaware) was wrapped around each kidney so that its entire surface was in contact with cellophane. The cellophane-wrapped kidney was enclosed in a polyethylene envelope and returned to the retroperitoneal space.

Controls

We did not manipulate the animals in the control group.

Measurement of Arterial Pressure by Percutaneous Arterial Catheter under Ketamine Immobilization

Animals were fasted for 14 to 16 hours and were immobilized with one intramuscular injection of ketamine (15 mg/kg). An 18-gauge Teflon catheter (Becton, Dickinson and Company, Rutherford, New Jersey) connected to a transducer (Statham P23D, Gould Incorporated, Cleveland, Ohio) was inserted in the left femoral artery as quickly as possible. Animals were positioned unrestrained on their left side, and blood pressure was charted on a recorder (Model R411, Beckman Instruments, Incorporated, Houston, Texas) that had been precalibrated against a mercury manometer.

We previously had compared blood pressure measured this way with pressure measured on teth

er (see following paragraph). We concluded that blood pressure obtained by percutaneous arterial
catheterization under these controlled conditions was satisfactory for classifying animals with high and low arterial pressures.

**Measurement of Arterial Pressure on Tether**

The tether system, modified from the apparatus described by Byrd, permitted continuous recording of arterial blood pressure of the conscious, unrestrained animal. We supplemented the pressures measured periodically during the experiment under ketamine immobilization with arterial pressures measured by the tether system near the termination of the experiment. Under anesthesia identical to that used for inducing renal artery stenosis, a midline abdominal incision extending from the umbilicus to the pubis provided access to the iliac arteries and femoral veins. An arterial catheter (Tygon Microbore Formula S-54-HL, Norton tubing; ID, 0.02 mm; OD, 0.17 mm; Scientific Products, Grand Prairie, Texas) was inserted through the wall of the internal iliac artery about 2 cm from its origin from the common iliac. The catheter’s tip lay just below the aortic bifurcation. A similar catheter was inserted into the femoral vein near the iliac bifurcation. The ends of the catheters were led cephalad along the dorso-lateral abdominal wall to the last rib, and then tunneled through muscles to an exit site over the 7th thoracic vertebra.

A polypropylene backpack held by dacron harness straps contained a transducer (Micron Model MP-15, Micron Instruments, Incorporated, Los Angeles, California) which was connected to the arterial catheter. A stainless steel, flexible tube connected the backpack to an electrical slip ring assembly (Airflyte Electronics, Bayonne, New Jersey) above the cage. The transducer was connected to a recording instrument and the venous catheter, to a bag of heparinized saline.

Ten days after implanting the catheters, we recorded the blood pressure (Model 2007-8686-00, Gould, Incorporated, Cleveland, Ohio) for 5 consecutive minutes out of every hour for 5 consecutive days. The recorder was calibrated to the transducer with a mercury manometer 2 days before recording of the blood pressure. Recording equipment was checked for machine drift several times each day and the transducer was recalibrated 2 days after the last recording. If the drift exceeded 10 mm Hg over the 9-day interval, the recording was discarded.

In a preliminary analysis, we determined that measurements every other hour provided an adequate summary of blood pressure. From the strip charts, we measured systolic and diastolic pressures for even-numbered hours with a digitizer (Model 9874A Digitizer, Hewlett Packard Company, Palo Alto, California). The blood pressures were grouped by “weekday daytime” (6 AM to 6 PM, Wednesday-Friday), “weekday nighttime” (6 PM to 6 AM), “weekend daytime” (Saturday and Sunday), and “weekend nighttime” for statistical analysis.

**Measurement of Heart Rate on Tether**

The frequency of the incoming pressure wave form was converted to beats per minute by a pressure processor amplifier. The recorded rate signal was digitized and the preset baseline was subtracted.

**Necropsy**

Twelve months after the operative procedure to produce hypertension and 10 months after beginning the atherogenic diet, we performed complete necropsies after immobilizing the animals with ketamine and pentobarbital and exsanguinating them through a venous catheter. We weighed and examined all major viscera. We dissected the coronary arteries from the heart, removed the atria, and cut the lateral right and left ventricular walls from the interventricular septum in the planes of the septal surfaces. We then weighed and measured the thicknesses of the septum and of each lateral wall.

We split the aorta into halves along the anterior and posterior midlines. The right half was fixed in 10% buffered formalin with its adventitial surface adherent to chipboard. We also opened and fixed in a similar manner the right iliac-femoral, brachial, and carotid arteries, and all three coronary arteries. The fixed arteries were then stained with Sudan IV and packaged in plastic bags.

**Grading Atherosclerosis**

Two pathologists experienced in grading arteries (Henry C. McGill, Jr., and Jack P. Strong, New Orleans, Louisiana) evaluated the atherosclerotic lesions independently. They estimated the percentage of surface area involved by all lesions and then estimated the percentage of lesion area involved with fatty streaks or fibrous plaques. Definitions of lesions were identical to those used in grading human lesions.

Fatty streaks were defined as Sudan IV-stained intimal areas that were elevated only slightly or not at all above the surrounding intimal surface. Fibrous plaques were defined as firm, distinctly elevated areas regardless of whether their intimal surface was stained for lipid. We prepared light and electron microscopic sections of representative lesions for examination. Intraclass correlation coefficients between the two pathologists in grading total involvement ranged from 0.74 in the abdominal aorta to 0.94 in the iliac-femoral artery. These correlation coefficients indicated acceptable agreement between the two pathologists. For all arteries except the coronary arteries, the mean of the two values was used in the statistical analyses. For coronary artery lesions, and for fibrous plaques in all arteries, the grades were converted to present or absent for statistical analyses, and group values were expressed as prevalence (proportion with lesions).
Statistical Analyses for Comparison of Treatment Groups

Each variable was analyzed separately by analysis of variance (ANOVA). For organ weight and cardiac wall thickness, total body weight was included as a covariable. The ANOVA, means, and confidence intervals were based on Huber M-estimates (c = 1.2). The robust procedure is less sensitive to outliers in the data. Natural logarithmic transformations were applied as required to better satisfy the assumptions underlying the ANOVA. When there were multiple measurements over time, the means and slopes of the linear regression line were used in separate analyses. These analyses used weighted ANOVA, with the weights obtained from the variance of the estimates from the linear regression analysis. Prevalence of lesions was analyzed by using a chi-square test. Where the expected frequencies were too small for the chi-square test, the data were analyzed by Fisher's exact test for 2 x 2 tables.

Statistical Analyses for Relation of Predictor Variables to Lesions

To investigate the relationships of various physiologic variables to atherosclerotic lesions, we divided the observations on the animals into "predictor" variables and "response" variables. The response variables were the percentage of intimal surface area involved with lesions (both fatty streaks and fibrous plaques). The predictor variables were treatment group, serum lipid concentrations, blood pressures, PRA, and serum electrolyte concentrations. The predictor variables represented an average of all observations of a variable for a particular animal. To develop prediction equations, we used multiple linear regression for each response variable separately. Where selection of variables was required, we used a forward selection procedure. The significance of added variables was judged by using the nominal significance levels. The order in which variables were included in the prediction equations is described with the results of this procedure.

Results

Blood Pressure

Table 3 shows arterial blood pressures averaged over six measurements made by percutaneous arterial catheter under ketamine immobilization before surgery and averaged over seven measurements between 4 and 54 weeks after surgery; also shown are arterial blood pressures and heart rates in the same groups measured by tether (without drug im...
Table 4. Mean Concentrations (95% Confidence Intervals) of Selected Plasma Components

<table>
<thead>
<tr>
<th>Material</th>
<th>Control</th>
<th>Renal artery stenosis</th>
<th>Bilateral perinephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity (ng/ml/hr)</td>
<td>15.3 (12.0–19.6)</td>
<td>28.5 (22.0–36.9)</td>
<td>16.1 (12.0–21.6)</td>
</tr>
<tr>
<td>Sodium (mEq/liter)</td>
<td>144 (143–144)</td>
<td>144 (143–144)</td>
<td>143 (142–144)</td>
</tr>
<tr>
<td>Potassium (mEq/liter)</td>
<td>3.2 (3.0–3.3)</td>
<td>2.9 (2.7–3.0)</td>
<td>3.3 (3.1–3.5)</td>
</tr>
<tr>
<td>Chloride (mEq/liter)</td>
<td>108 (107–109)</td>
<td>106 (104–107)</td>
<td>108 (107–110)</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>13.9 (12.8–15.1)</td>
<td>16.1 (14.8–17.4)</td>
<td>14.3 (12.9–15.7)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.3 (1.2–1.4)</td>
<td>1.6 (1.5–1.7)</td>
<td>1.4 (1.3–1.5)</td>
</tr>
<tr>
<td>VLDL + LDL cholesterol (mg/dl)</td>
<td>86 (70–105)</td>
<td>103 (85–126)</td>
<td>99 (80–124)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>120 (109–132)</td>
<td>123 (112–135)</td>
<td>110 (99–122)</td>
</tr>
</tbody>
</table>

Cholesterol was determined by the heparin-manganese precipitation method (see reference 21).

mobilization or physical restraint) during a 1-week period 5 weeks before necropsy. Average systolic and diastolic pressures measured under ketamine were equal among the groups before surgery. Renal artery stenosis elevated both systolic (45 mm Hg) and diastolic (34 mm Hg) pressures; and perinephritis elevated pressures somewhat less (systolic, 20 mm Hg; diastolic, 19 mm Hg). Pressures of both treatment groups were significantly greater than those of the control group (p < 0.01), and renal artery stenosis pressures were significantly greater than the perinephritis pressures (p < 0.02).

Blood pressures measured by tether without anesthesia during the daytime were similar to those measured under ketamine. Tether pressures were significantly lower at night (p < 0.05). However, the renal artery stenosis and bilateral perinephritis groups were equal to one another, and both were higher than the control group (p < 0.01) in both daytime and nighttime pressures. Heart rate was significantly higher (p < 0.05) in the renal artery stenosis group compared to the control group.

Renin Activity, Electrolytes, Urea Nitrogen, Creatine, and Lipoprotein Cholesterol

Table 4 summarizes these values averaged over the entire experimental period. As anticipated, PRA in the renal artery stenosis group was about double that in the control and bilateral perinephritis groups (p < 0.01); potassium and chloride levels were lower in the renal artery stenosis group (p < 0.01). There were no differences in sodium concentrations. The renal artery stenosis group had a slightly higher concentration of urea nitrogen (p < 0.025) and creatinine (p < 0.01) than the control group. There were no significant differences in VLDL-C + LDL-C or HDL-C among the three groups.

Although the trend was not statistically significant, PRA declined during the experiment. The decline was greatest in the renal artery stenosis group, but appeared to level off during the last 4 months of the experiment. During the last 4 months, PRA remained significantly higher in the renal artery stenosis group than in the other groups (control, 13.3 ng/ml/hr; renal artery stenosis, 20.9 ng/ml/hr; and bilateral perinephritis, 13.5 ng/ml/hr).

Other Comparisons

There were no significant differences among the three groups in white blood cell count or hematocrit, hemoglobin concentration, serum total protein or albumin concentrations, or glutamic-oxaloacetic-transaminase or glutamic-pyruvate-transaminase activities (results not shown).

Table 5. Cardiac and Ventricular Weight and Ventricular Thickness (95% Confidence Intervals) Adjusted for Total Body Weight

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Renal artery stenosis</th>
<th>Bilateral perinephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart, weight (g)</td>
<td>123 (114–132)</td>
<td>122 (113–130)</td>
<td>133 (123–143)</td>
</tr>
<tr>
<td>Right ventricle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>25.8 (24.0–27.6)</td>
<td>22.9 (20.9–24.9)</td>
<td>25.5 (23.4–27.6)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>4.6 (4.2–4.9)</td>
<td>4.2 (3.9–4.6)</td>
<td>4.2 (3.8–4.7)</td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>46.5 (42.1–51.0)</td>
<td>46.4 (41.5–51.4)</td>
<td>45.9 (39.9–52.0)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>12.1 (11.3–12.8)</td>
<td>11.6 (10.8–12.4)</td>
<td>12.0 (11.3–12.6)</td>
</tr>
<tr>
<td>Interventricular septum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>35.7 (32.4–39.1)</td>
<td>34.1 (30.3–37.8)</td>
<td>41.8 (37.3–46.4)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>10.7 (10.1–11.3)</td>
<td>10.4 (9.8–11.0)</td>
<td>12.0 (11.3–12.6)</td>
</tr>
</tbody>
</table>
Table 6. Mean Percentage of Surface Involved by All Lesions (95% Confidence Intervals) by Artery

<table>
<thead>
<tr>
<th>Artery</th>
<th>Control</th>
<th>Renal artery stenosis</th>
<th>Bilateral perinephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>18.8 (9.7–27.8)</td>
<td>21.2 (12.4–30.0)</td>
<td>18.4 (8.4–28.4)</td>
</tr>
<tr>
<td>Thoracic</td>
<td>11.6 (2.8–20.4)</td>
<td>26.7 (18.0–35.3)</td>
<td>23.2 (13.5–33.0)</td>
</tr>
<tr>
<td>Abdominal</td>
<td>8.5 (1.1–16.0)</td>
<td>22.6 (15.3–29.8)</td>
<td>13.7 (5.9–21.4)</td>
</tr>
<tr>
<td>Iliac-femoral</td>
<td>5.6 (1.4–9.9)</td>
<td>13.5 (9.4–17.7)</td>
<td>11.9 (7.2–16.6)</td>
</tr>
<tr>
<td>Brachial</td>
<td>5.4 (0.0–12.4)</td>
<td>21.9 (15.1–28.8)</td>
<td>15.2 (7.5–22.9)</td>
</tr>
</tbody>
</table>

Heart Size

There were no significant differences among the groups in total heart weight, right ventricular weight and thickness, or left ventricular weight and thickness (Table 5). The interventricular septum was heavier ($p < 0.05$) and thicker ($p < 0.01$) in the bilateral perinephritis group than in the control or renal artery stenosis groups, but the difference was not large. We concluded that neither form of experimental hypertension produced an appreciable degree of cardiac hypertrophy or dilatation.

Characteristics of Lesions

Light microscopy of representative lesions confirmed that those classified grossly as fatty streaks were composed principally of lipid in macrophages, smooth muscle cells, and intercellular spaces of the intima. There was only slight thickening of the intima. In contrast, lesions classified grossly as fibrous plaques showed an intimal layer thickened predominantly by smooth muscle cells but also containing extracellular lipid globules together with crystalline structures. A moderate number of macrophages both with and without lipid were present in both fatty streaks and fibrous plaques, but the lesions were not predominantly composed of foam cells. Electron micrographs confirmed the presence of these lesion elements. Comparison of lesions in hypertensive animals with those in nonhypertensive animals showed no qualitative differences between the two.

Atherosclerotic Lesions by Treatment Group

The hypertensive animals, compared to the controls, had approximately double the extent of involvement with atherosclerotic lesions in the abdominal aorta, iliac-femoral artery, and brachial artery, and approximately triple the extent in the carotid artery (Table 6). The mean value for these arteries of each hypertensive group was significantly ($p < 0.05$) different from the corresponding value of the control group, with the one exception that for the iliac-femoral artery, the bilateral perinephritis group was not different from the control group. There was no difference among the three groups in lesions of the thoracic aorta. Comparison of the groups by analysis of covariance (ANCOVA) with VLDL-C + LDL-C and HDL-C as covariates yielded results similar to those in Table 6.

The prevalence of lesions in the coronary arteries was increased to about the same degree ($p < 0.05$) in both hypertensive groups as compared to the control group (Table 7).

The prevalence of fibrous plaques was increased in most of the arteries, but the increases were not statistically significant (Table 8).

Relationship of Variables to Atherosclerotic Lesions

In developing the prediction equations, we included first the VLDL-C + LDL-C and HDL-C concentrations because, in a previous experiment, these had been good predictors. We next included a blood pressure variable. Based on the order of selection across all arteries, we selected the systolic blood pressure measured with the tether and averaged across weekday, weekend, day, and night. VLDL-C + LDL-C was the most consistent predictor of lesions. HDL-C coefficients were generally small but of primarily negative sign. The coefficient for blood

Table 7. Prevalence of Lesions in Coronary Arteries (95% Confidence Intervals)

<table>
<thead>
<tr>
<th>Coronary artery</th>
<th>Control</th>
<th>Renal artery stenosis</th>
<th>Bilateral perinephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>0.00 (0.00–0.21)</td>
<td>0.38 (0.15–0.65)</td>
<td>0.31 (0.09–0.61)</td>
</tr>
<tr>
<td>Left circumflex</td>
<td>0.09 (0.00–0.21)</td>
<td>0.47 (0.21–0.73)</td>
<td>0.46 (0.19–0.75)</td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>0.35 (0.14–0.62)</td>
<td>0.71 (0.44–0.96)</td>
<td>0.71 (0.42–0.92)</td>
</tr>
</tbody>
</table>
pressure was larger than its standard error for all arteries except the thoracic aorta. Variables representing the interaction of lipoprotein cholesterol and blood pressure did not add significantly to the $R^2$. The multiple correlation coefficient squared, $R^2$, indicates the proportion of variability in the response variable that is explained by the predictor variables. When we added variables to represent the three treatment groups to the predictor variables VLDL-C + LDL-C, HDL-C, and blood pressure, the $R^2$ ($R^2_b$, Table 9) remained about the same for all arteries except the carotid artery, for which it was increased ($p < 0.05$). For the arteries in which $R^2$ did not change, the similarity suggests that the method of inducing hypertension affected lesions primarily by elevating blood pressure. For the carotid artery, in which $R^2$ was increased, the method of inducing hypertension affected lesions partly but not exclusively by elevating blood pressure.

We classified the coronary arteries as positive when the average of the grades of the two pathologists was greater than 0.5% of the surface area involved. The results of multiple logistic function analyses of predictor variables and coronary artery lesions are shown in Table 10. VLDL-C + LDL-C and systolic blood pressure gave consistently positive coefficients, and HDL-C gave negative coefficients for two of the three branches.

The heart rate was correlated with blood pressure ($r = 0.44$, $p < 0.05$, partial correlation, adjusted for treatment group). When added to multiple regression equations after lipoprotein cholesterol concentrations and blood pressure, the heart rate did not significantly increase $R^2$ in any of the arteries.

To investigate potential mechanisms by which the method of inducing hypertension affected lesions other than by increasing blood pressure, we used serum potassium concentration as a predictor vari-

### Table 9. Multiple Regression Coefficients for Lipoprotein Cholesterol and Blood Pressure for Extent of All Lesions by Artery

<table>
<thead>
<tr>
<th>Artery</th>
<th>No.</th>
<th>Intercept</th>
<th>VLDL-C + LDL-C</th>
<th>HDL-C</th>
<th>Systolic blood pressure</th>
<th>$R^2_b$</th>
<th>$R^2_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoracic</td>
<td>41</td>
<td>31.2</td>
<td>0.20†</td>
<td>-0.22*</td>
<td>-0.04</td>
<td>0.24</td>
<td>0.25</td>
</tr>
<tr>
<td>Abdominal</td>
<td>41</td>
<td>-13.4</td>
<td>0.06*</td>
<td>0.04</td>
<td>0.17*</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Iliac-femoral</td>
<td>40</td>
<td>-28.1</td>
<td>0.02</td>
<td>-0.07*</td>
<td>0.34*</td>
<td>0.49</td>
<td>0.51</td>
</tr>
<tr>
<td>Brachial</td>
<td>41</td>
<td>-11.8</td>
<td>0.10†</td>
<td>-0.03</td>
<td>0.11*</td>
<td>0.31</td>
<td>0.37</td>
</tr>
<tr>
<td>Carotid</td>
<td>41</td>
<td>-31.6</td>
<td>0.18†</td>
<td>-0.06</td>
<td>0.24†</td>
<td>0.53</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Numbers in parentheses are standard errors.

*Coefficient divided by se $> 1$.
†Coefficient divided by se $> 2$.
‡$R^2_b$: predictor variables are VLDL-C + LDL-C, HDL-C, and systolic blood pressure; $R^2_b$: when experimental group was added.
### Table 10. Multiple Logistic Function Coefficients for Lipoprotein Cholesterol and Blood Pressure for Presence of Coronary Artery Lesions

<table>
<thead>
<tr>
<th>Coronary Artery</th>
<th>Proportion Positive</th>
<th>Intercepts</th>
<th>VLDL-C + LDL-C</th>
<th>HDL-C</th>
<th>Systolic Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>10/38</td>
<td>-6.17</td>
<td>0.02*</td>
<td>-0.03*</td>
<td>0.04†</td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>19/41</td>
<td>-2.66</td>
<td>0.01*</td>
<td>-0.02*</td>
<td>0.03*</td>
</tr>
<tr>
<td>Circumflex</td>
<td>9/37</td>
<td>-10.13</td>
<td>0.02*</td>
<td>-0.00</td>
<td>0.04†</td>
</tr>
</tbody>
</table>

Numbers in parentheses are standard errors.
*Coefficient divided by its SE ≥ 1.
†Coefficient divided by its SE ≥ 2.

able (Table 11). The coefficient for serum potassium concentration was negative for all arteries and was about three times its standard error for the carotid artery. Its inclusion significantly increased the $R^2$ ($R^2_b$) for the carotid artery. Inclusion of the experimental group variables added only slightly to the $R^2$ ($R^2_b$ compared to $R^2_a$).

Because of a laboratory failure, PRA measurements were not obtained on approximately one-fourth of the animals. When added to lipoprotein cholesterol and blood pressure measurements, the coefficients for PRA were positive and were larger than their standard errors for all arteries except the iliac-femoral (Table 12). The increase in $R^2$ ($R^2_b$ compared to $R^2_a$) when PRA was added to lipoprotein cholesterol and blood pressure was significant for the carotid artery. Little additional predictive capability was added by including both potassium and PRA when compared to PRA alone, or by adding experimental group variables.

For the carotid artery, the systolic blood pressure measured under ketamine and averaged over the experiment was a better predictor than systolic blood pressure measured with the tether ($R^2 = 0.56$ when using VLDL-C + LDL-C, HDL-C, and blood pressure). When serum potassium concentration was added, the increase in $R^2$ was significant. When PRA was added, the increase in $R^2$ was not significant.

### Discussion

**Comparison with Other Studies**

The results clearly confirm those of previous experiments in which hypertension augmented experimental atherosclerosis in dogs, rats, rabbits,
Table 12. Multiple Regression Coefficients for Lipoprotein Cholesterol, Blood Pressure, and Plasma Renin Activity for Extent of All Lesions by Artery

<table>
<thead>
<tr>
<th>Artery</th>
<th>No.</th>
<th>Intercept</th>
<th>VLDL-C + LDL-C</th>
<th>HDL-C</th>
<th>Systolic Blood Pressure</th>
<th>Plasma Renin Activity</th>
<th>Squared multiple correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>29</td>
<td>-12.5</td>
<td>0.24† (0.17)</td>
<td>-0.10</td>
<td>0.11* (0.02)</td>
<td>0.23* (0.22)</td>
<td>0.42</td>
</tr>
<tr>
<td>Thoracic</td>
<td>29</td>
<td>-60.4</td>
<td>0.10* (0.07)</td>
<td>0.20* (0.17)</td>
<td>0.31† (0.10)</td>
<td>0.29* (0.23)</td>
<td>0.42</td>
</tr>
<tr>
<td>Abdominal</td>
<td>28</td>
<td>-29.5</td>
<td>0.05 (0.06)</td>
<td>-0.11</td>
<td>0.37† (0.08)</td>
<td>0.02</td>
<td>0.53</td>
</tr>
<tr>
<td>Iliac-femoral</td>
<td>29</td>
<td>-36.1</td>
<td>0.14† (0.04)</td>
<td>0.07 (0.10)</td>
<td>0.17† (0.06)</td>
<td>0.18* (0.14)</td>
<td>0.52</td>
</tr>
<tr>
<td>Brachial</td>
<td>29</td>
<td>-47.5</td>
<td>0.24† (0.04)</td>
<td>-0.01</td>
<td>0.24‡ (0.11)</td>
<td>0.35† (0.15)</td>
<td>0.68</td>
</tr>
<tr>
<td>Carotid</td>
<td>29</td>
<td>-47.5</td>
<td>0.24† (0.04)</td>
<td>-0.01</td>
<td>0.24‡ (0.11)</td>
<td>0.35† (0.15)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Numbers in parentheses are standard errors.
*Coefficient divided by SE ≥ 1.
†Coefficient divided by SE ≥ 2.
‡R², predictor variables are VLDL-C + LDL-C, HDL-C, systolic blood pressure; R², when plasma renin activity was added.

Comparing Two Types of Hypertension

Renal artery stenosis doubled PRA but bilateral perinephritis did not alter PRA (Table 4). These two hypertensive groups had similar average blood pressure elevations at the end of the experiment (Table 3). The renal artery stenosis group had a greater extent of atherosclerotic lesions and a higher prevalence of fibrous plaques than the bilateral perinephritis group in all arteries (with the one exception of fibrous plaques in the iliac-femoral artery), but the differences were not statistically significant. The results examined this way do not suggest that PRA significantly influences atherogenesis.

Multiple Regression Analysis of Predictor Variables

There also was considerable variation in PRA among individual animals of all three groups. Multiple regression analysis showed that lipoprotein cholesterol and blood pressure were strong predictors of atherosclerotic lesions, as anticipated (Tables 9 and 10). However, when either potassium concentration or PRA was added to the regression equation, the increase in R² was statistically significant (p < 0.05) in the carotid arteries (Tables 11 and 12), where the augmentation of atherogenesis associated with hypertension was most marked.

The group comparisons showed differences in lesions parallel to those in PRA and potassium (Tables 6–8), but these differences were not statistically significant. Multiple regression analysis takes into account individual variations in PRA and potassium concentration as well as individual variations in lipoprotein cholesterol and blood pressure. The results suggest that either PRA, or potassium, or both (or some variable other than blood pressure that is closely related to PRA and potassium) are associated with the rate of atherogenesis in some arteries beyond the effect of blood pressure alone. However, as in all exploratory data analyses, this approach does not provide a critical test of the question of the role of PRA above that of blood pressure.

Interrelationships of Plasma Renin Activity and Serum Potassium Concentration

Plasma renin activity and serum potassium concentration were inversely correlated with one another in this experiment (r = −0.47, partial correlation, adjusted for treatment group). The inverse correlation is reflected by the positive (PRA) and negative (potassium) multiple regression coefficients for atherosclerotic lesions. These findings also are consistent with the inverse relationship between serum potassium concentration and PRA observed in both humans and experimental animals. It is believed that elevated serum potassium lowers renin secretion by increasing the delivery of sodium to the macula densa, which regulates renin release from the juxtaglomerular apparatus. These results provide no information about whether the physiological mechanism affecting atherogenesis is based on serum potassium concentration, PRA, or related vasooactive hormones.

bits, and monkeys. They also are consistent with the many observations that human hypertension is associated with more severe atherosclerosis. However, the results are not consistent with other experiments that have examined the relationship of PRA to atherogenesis.

Comparing Two Types of Hypertension

Renal artery stenosis doubled PRA but bilateral perinephritis did not alter PRA (Table 4). These two hypertensive groups had similar average blood pressure elevations at the end of the experiment (Table 3). The renal artery stenosis group had a greater extent of atherosclerotic lesions and a higher prevalence of fibrous plaques than the bilateral perinephritis group in all arteries (with the one exception of fibrous plaques in the iliac-femoral artery), but the differences were not statistically significant. The results examined this way do not suggest that PRA significantly influences atherogenesis.

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Other Experiments On Plasma Renin Activity

Overturf and associates conducted the most direct and extensive experimental test of the hypothesis that renin accelerates atherogenesis. Two-kidney, one clip (high renin) hypertension and one-kidney, one clip (low renin) hypertension augmented to the same degree experimental atherosclerosis in hypercholesterolemic rabbits and fibromuscular intimal plaques in normocholesterolemic rabbits. Deoxycorticosterone-saline treatment lowered PRA but did not elevate blood pressure and did not influence cholesterol-induced atherosclerosis. These experiments, therefore, did not demonstrate either acceleration of atherogenesis by elevated PRA or retardation by lowered PRA.

The present experiment differs from those reported by Overturf and associates in two ways other than in species. The principal difference is the level of hypercholesterolemia: the cholestrol-fed rabbits averaged 600 mg/dl to 1000 mg/dl total serum cholesterol, predominantly carried in VLDL-C + LDL-C, while the baboons averaged about 200 mg/dl, with VLDL-C + LDL-C/HDL-C ratios of about 0.8. The high levels of lower density lipoproteins in the hypercholesterolemic rabbits may have masked the effects of other modulating factors.

The second difference is in the arterial segments compared. In rabbits, the lesions were most extensive in the aortic arch and less extensive in the thoracic and abdominal segments, and the comparison was based mainly on thoracic aorta lesions. In baboons, thoracic aorta lesions were not affected by hypertension, whereas carotid artery lesions were affected by blood pressure, PRA, and serum potassium concentration.

In a small number of normotensive stump tail macaques (Macaca arctoides) with plasma cholesterol levels ranging from about 500 mg/dl to 700 mg/dl, Pick and Glick found no effect of minoxidil (an anti-hypertensive drug that increases PRA) on experimental atherosclerosis. In another experiment with stump tail macaques with similar plasma cholesterol levels and renal hypertension, Pick et al. treated some groups with a variety of antihypertensive agents but found no effect of the antihypertensive treatment on experimental atherosclerosis.

Interaction with Hyperlipidemia

Hypertension induces many alterations in the large muscular and elastic arteries in the absence of hyperlipidemia. It increases the extent of muscular-elastic intimal plaques in rhesus monkeys, increases medial wall thickness, increases endothelial cell proliferation, occasionally produces endothelial denudation, stimulates intimal accumulation in the acute phase of hypertension but not in the chronic phase, alters endothelial structure, alters endothelial gap junctions, increases aortic connective tissue, and increases in vitro cholesterol-biosynthesis. Schwartz compared the effects of hypertension and atherosclerosis on both endothelial and smooth muscle cell replication. However, hypertension usually does not induce lesions typical of atherosclerosis (that is, a combination of lipid deposition, macrophage accumulation, and smooth muscle and connective tissue proliferation) unless it is combined with an elevation in serum lipids and lipoproteins. Because we did not include a chow-fed control group in this experiment, the range of hyperlipidemia was limited to the naturally occurring variations in response to the atherogenic diet. VLDL-C + LDL-C and HDL-C remained strong predictors of atherosclerosis, but these analyses did not indicate a statistically significant interaction between hyperlipidemia and hypertension in their effects on atherosclerosis. The lack of such an interaction is probably due to the limited range of hyperlipidemia.

Conclusion

The results of this experiment confirm the well-established atherogenic effects of hypertension in the presence of moderate hyperlipidemia and emphasize the selectivity of its effect on the carotid arteries. Our observations suggest that some factor in renal hypertension associated with the vasoactive hormones affects atherogenesis in addition to blood pressure, particularly in the carotid arteries.

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Jack P. Strong, Department of Pathology, Louisiana State University Medical Center, New Orleans, graded the arteries. Frederic C. Bartter (deceased), Associate Chief of Staff for Research and Development, Audie L. Murphy Memoral Veterans Administration Hospital, San Antonio, Texas, supervised the measurements of plasma renin activity and serum electrolytes.

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Index Terms: hypertension • plasma renin activity • atherosclerosis • baboon
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H C McGill, Jr, K D Carey, C A McMahan, Y N Marinez, T E Cooper, G E Mott and C J Schwartz

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