The sympathetic nervous system and atherosclerosis

TERRY LICHTOR, M.D., PH.D., HARRY R. DAVIS, PH.D., LYDIA JOHNS, DRAGA VESSELINEVITCH, D.V.M., ROBERT W. WISSLER, M.D., PH.D., AND SEAN MULLAN, M.D.

Section of Neurological Surgery and Department of Pathology, The University of Chicago Medical Center, Chicago, Illinois

Morphometric and chemical changes in the arterial wall were studied after 12 months of diet-induced atherosclerosis in rhesus monkeys treated with either bilateral surgical thoracic sympathectomy or propranolol. There was a marked reduction in the progression of atherosclerosis in the carotid arteries and a moderate reduction in the disease found in the thoracic aorta of monkeys treated initially with a sympathectomy, in comparison to control monkeys fed an atherogenic diet alone. Propranolol at a dose of 40 mg/12 hrs also seemed to reduce the progression of atherosclerosis in the carotid arteries and thoracic aorta, although the differences were less dramatic. There were minimal differences in the extent of atherosclerosis in the abdominal aorta or femoral arteries of animals in either treatment group as compared with the control group. Similarly, the chemical composition of these same major vessels showed no significant differences. Therefore, in the face of severe atherogenic stimuli, chemical or surgical sympathectomy may be useful in controlling atherosclerosis in specific arterial beds.

KEY WORDS • thoracic sympathectomy • propranolol • atherogenesis • atherosclerosis • rhesus monkey

The major risk factors for coronary atherosclerosis are hyperlipidemia, smoking, and hypertension. Behavioral factors have also been implicated in both human and nonhuman primate studies. Among behavioral factors of potential importance in humans, the well-described coronary-prone behavior pattern has attracted considerable interest and has also been the subject of extensive epidemiological investigation. The classical coronary-prone (Type A) individual responds to his environment with a sense of time urgency, intense competitiveness, and poorly modulated hostility. The relative absence of these attributes denotes noncoronary-prone behavior. Although the findings are somewhat inconclusive, several groups of investigators have reported greater severity of coronary atherosclerosis among these Type A individuals as recorded on angiographic examination and at autopsy. The established risk factors probably account for the major proportion of patients with atherosclerotic heart disease; nevertheless there is a proportion of subjects who develop this disease in the apparent absence of the major risk factors or genetic predisposition.

In studies of experimental atherosclerosis in cynomolgus monkeys, response to social stress has been implicated even in the face of normal serum lipid profiles. In addition, cynomolgus monkeys showing a high heart-rate reactivity to stress developed more atherosclerosis in the face of a moderately atherogenic diet. In another study, Beere, et al., have shown that a steady-state heart rate is inversely correlated with the degree of coronary atherosclerosis in cynomolgus monkeys with severe diet-induced atherosclerosis. The mediators of these behavioral effects and of heart-rate reactivity may be the sympathetic nervous system and catecholamine receptors in the cardiovascular system.

In spite of extensive experience with surgical sympathectomy, both lumbar and cervical, a definite therapeutic effect on atherosclerosis has not been established. A response could be masked by the rather late application of this treatment when chronic scarring and calcification of atherosclerotic lesions are long established. It could also be overridden by a diet with an overwhelming atherogenic component. The general clinical impression has been that sympathectomy does have some ameliorating value in terms of distal ischemia by releasing functional vessels, but that progressive arterial narrowing would soon negate this improvement. There is even some experimental evidence that sympathectomy supports increased atherosclerotic progression. In 1956, Murphy, et al., found no athero-
Sympathectomy and atherosclerosis

Sclerosis in control rabbits subjected to bilateral lumbar sympathectomy with periaortic stripping of all sympathetic connections nor in those fed a high-fat and cholesterol diet. However, in rabbits subjected to a combination of the high-fat and cholesterol diet and the surgical lesion, they found extreme lower lumbar and femoral atherosclerosis. Certainly, the damage inherent in the periaortic stripping makes the interpretation of these experiments somewhat difficult. In 1958, Snyder and Campbell confirmed Murphy's observations in the lumbar and iliac arteries and in the aorta. In 1968, Marinescu, et al., carried out extensive lumbar sympathectomy in dogs and reported metabolic disorders in the arterial walls, involving mainly protein metabolism. Furthermore, aggravation of previous lesions was noted in some of the animals, which in a few instances resulted in irreversible vascular obstruction. The lipid content of the vessels was not examined in this study, and the observations were made after a relatively short interval of between 20 and 50 days.

In rabbits and rats subjected to chemical sympathectomy (6-hydroxydopamine), Fronek, et al., found that changes compatible with an aging process occur in collagen synthesis (250% higher) and in the elastic elements within the media. The vessels showed increased sensitivity to circulating adrenaline. With the addition of 1% cholesterol to the diet in the rabbit model there was an even greater increase in lipid levels of the aortic wall in the sympathectomized animals than in the control animals. It was concluded that sympathectomized arteries are more susceptible to atherosclerosis in the presence of a high cholesterol level.

In 1971, Austin, et al., subjected rabbits to a high-cholesterol diet and carried out a unilateral sympathectomy. In this study more lipid deposits were found in the iris of the sympathectomized eye than in the iris of the unoperated side. The lipid was in the smooth muscle cells, as is true in atherosclerotic vessels. A high level of correlation was found between the deposition of lipid in the iris and in the aorta.

In 1973, Whittington-Coleman, et al., evaluated the effect of propranolol (5 mg daily) in rabbits. Animals on a high-cholesterol diet with propranolol had profoundly reduced fat deposition along the intimal layer and inside the endothelial cells of the aorta, despite a significantly higher serum cholesterol level than that of animals not given propranolol. These results were confirmed in a similar study by Chobanian, et al., in 1985. In 1977, Pick and Glick carried out a pilot study of four stumptail macaques, two of which were given propranolol (40 mg/8 hrs) in addition to the atherogenic diet. The serum cholesterol level increased in all four. The "aortic grade" of lesions in the propranolol-treated animals was about half that in the control group, and the "coronary index," which combines involvement and severity, was only about 20% the value in control animals. A more detailed study was then made of 10 stumptail macaques, again administering 120 mg propranolol daily. All animals had increased plasma cholesterol levels. It was discovered, however, that there was no difference in "aortic grade" or in "coronary index" between the propranolol-treated and the control monkeys (both on atherogenic diets) after 6 months. There is no explanation for the discrepancy between the pilot and the definitive studies.

In summary, there is no consensus in the literature as to the relationship between atherosclerosis and the sympathetic nervous system. In this study, rhesus monkeys were used to further investigate the effects of surgical and chemical sympathectomy on the progression of atherosclerosis. The long-term objectives seek to modify the development of atherosclerosis in the human by manipulation of the stress-transmitting sympathetic system and its receptors (as well as by manipulation of the well-known risk factors of cholesterol, hypertension, and cigarette smoking).

Materials and Methods

Experimental Design

The experimental design of this study is summarized in Table 1. Young adult male rhesus monkeys were used in this study. At the beginning of the experiment, they were divided into three groups with comparable distribution of body weights and baseline serum cholesterol levels. Group 1 (10 animals) underwent bilateral thoracic sympathectomy prior to receiving the atherogenic diet. Group 2 (six animals) was fed propranolol* (40 mg/12 hrs) in addition to the atherogenic diet. Group 3 (seven animals) was fed the atherogenic diet alone. Due to the limited availability of animals for this study, more animals were assigned to the sympathectomy group than to the other two groups. This was done because we anticipated that some animals might die during or after surgery, but fortunately this did not happen. As we already have abundant previous experience and a large amount of quantitative information on rhesus monkeys fed this particular atherogenic diet, only a few monkeys were placed in the group receiving the atherogenic diet without treatment. All of the animals were observed in quarantine for 1 month before the beginning of the studies to insure animal health and normal laboratory values prior to the start of the diet.

* Propranolol supplied by Ayerst Laboratories, New York, New York.

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>No. of Animals</th>
<th>Experimental Procedure &amp; Dietary Regimen*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>bilateral sympathectomy + atherogenic diet</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>atherogenic diet + propranolol (40 mg/12 hrs)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>atherogenic diet</td>
</tr>
</tbody>
</table>

* For ingredients of the atherogenic diet see Table 2.
period. All animals were kept on the atherogenic diet for a period of 1 year. Serum for chemical analyses was obtained from the animals after they had been fasted overnight. The animals were bled twice prior to the dietary manipulation, then monthly for 2 months after diet initiation, and then bimonthly for the remainder of the study.

**Surgical Aspects**

For surgery, ketamine anesthesia and full sterile technique were used. A bilateral transthoracic sympathectomy at T1–6 and stellate ganglionectomy were carried out in two stages separated by 3 weeks. Three weeks later the atherogenic diet was started.

**Diet**

The atherogenic diet used in this experiment is outlined in Table 2. This diet has been shown to produce advanced atherosclerosis in rhesus monkeys in relatively brief periods of time, and the arterial lesions have been found to have many features similar to those seen in the human disease. A consistently formulated pulverized primate ration with no added animal fat serves as the base to which 2% cholesterol and 12.5% each of coconut oil and butterfat is added. The atherogenic ration yields 424 calories/100 gm. This ration, although richer in cholesterol than most human diets, is designed to mimic the highly saturated fat content frequently found in Western society diets. This diet is readily accepted by rhesus monkeys, and in general supports weight gain and a healthy appearance. The propranolol tablets were given with a piece of fruit with supervision to ensure that the tablets were actually swallowed.

**Animal Care**

The animals were first maintained in isolation and in quarantine for a period of 1 month until found to be free of evidence of disease such as tuberculosis, *Shigella*, or *Salmonella*. During that time they were tuberculin-tested three times, tattooed for permanent identification, and carefully examined for worms, parasites, and bacterial infections. The monkeys were then housed separately and cared for at the A. J. Carlson Animal Facility of the University of Chicago. The physical plant as well as animal maintenance and husbandry are in compliance with the Laboratory Animal Welfare Act, and we followed the principles for the use of animals as outlined by the National Institutes of Health (NIH Manual, Chapter 4206).

**Pathological Evaluation**

Standardized autopsy procedures were utilized to ascertain precise quantitative morphological data. The animals underwent autopsy immediately following exsanguination under ketamine and pentobarbital anesthesia. An arterial catheter was placed and blood pressure readings and heart rates were recorded following induction of anesthesia with ketamine and pentobarbital and before any blood was removed. The aorta and heart were first removed en bloc. The ascending aorta was then transected approximately 0.5 cm from the aortic valve. The aorta was next placed immediately on a 4°C surface and all adventitial fat was quickly removed. The aorta was then opened from its dorsal side with the ostia of the celiac, superior, and inferior mesenteric arteries and the renal arteries intact and exposed on the ventral and lateral surfaces. After opening the aorta, the surface involved grossly with lesions was measured and photographed. Carotid and femoral arteries were processed similarly. Samples of the arteries were obtained for microscopy, then the remainder were opened and the percent of surface area involved grossly with lesions was measured using a slight modification of the method developed by Howard. Two observers carried out this evaluation independently, and their results were subsequently averaged. Standardized anatomical samples of the remaining aorta were used for chemical analysis.

The aorta was trimmed in the following standard manner, so that weight and area comparisons could be made: from 1.5 cm above the aortic ring distally including 1.0 cm of the iliac arteries, and with 2.0 cm lengths of the innominate, subclavian, and carotid arteries attached. Anatomically defined samples for light microscopy were taken from each animal: adjacent to the ostium of the left subclavian artery sample (1A), 1 cm above the celiac artery sample (2A), from the abdominal aorta 3 cm below the renal arteries sample (3A), and from a point beginning 5 mm below the bifurcation of the iliac arteries sample (4A). The last sample (5A) was taken 5 cm distal to the ostium of the right innominate and the left common carotid arteries. The proximal blocks of all areas were used for fat stain with Oil Red O, while paraffin sections were prepared from each distal sample and stained for connective tissue.

---

**Table 2**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% Wet Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>coconut oil</td>
<td>12.5</td>
</tr>
<tr>
<td>butterfat</td>
<td>12.5</td>
</tr>
<tr>
<td>cholesterol</td>
<td>2.0</td>
</tr>
<tr>
<td>primate chow*</td>
<td>57.8</td>
</tr>
<tr>
<td>vitamin mixture†</td>
<td>0.9</td>
</tr>
<tr>
<td>gelatin</td>
<td>1.3</td>
</tr>
<tr>
<td>orange juice</td>
<td>13.0</td>
</tr>
<tr>
<td>total</td>
<td>100.0</td>
</tr>
<tr>
<td>calories (100 gm)‡</td>
<td>424</td>
</tr>
</tbody>
</table>

---

* Pulverized constant formula and constant ingredient primate ration with no animal fat added was obtained through special arrangement with the Ralston Purina Co.
† Vitamin mixture: ascorbic acid 50 gm; niacin 10.0 gm; vitamin A 6.0 gm; thiamin 2.0 gm; riboflavin 2.0 gm; pyridoxine 2.0 gm; calciferol 1.08 gm; folic acid 0.2 gm; biotin 0.04 gm; calcium pantothenate 6.0 gm; and sucrose 992.1 gm.
‡ The atherogenic diet has a caloric distribution of 14% protein, 55% carbohydrates, and 31% fat.
tissue elements with Gomori trichrome-aldehyde-fuchsin stain.

The carotid and femoral arteries were studied in a similar fashion. They were fixed in neutral buffered formalin as soon as possible after they were removed, and were sampled in a standard manner at two points (the proximal and distal ends of each artery) while the portion between the two points in both the carotid and the femoral arteries was used for chemical analysis.

The heart was fixed by pressure perfusion of the coronary arteries with phosphate-buffered 2.5% glutaraldehyde at a pressure of 90 mm Hg. The coronary arteries were sampled at nine standardized areas as described previously. Proximal sections of two blocks removed from each of nine specific areas were stained with Oil Red O for lipids, while the interfacing surfaces of the distal samples were stained with hematoxylin and eosin (H & E) and Gomori trichrome-aldehyde-fuchsin.

Paraffin sections of all formalin-fixed tissues were cut 4 μ thick and stained with H & E and also by the Gomori trichrome-aldehyde-fuchsin method for identification of cells, collagen, and elastin. Fixed frozen free-floating sections were cut 12 μ thick, stained with Oil Red O and Lillie-Mayer hematoxylin, and mounted on slides with neutralized glycerol gelatin. The paraffin sections were cover-slipped with Permount.

Biochemical Analyses of Tissue Samples

Standardized samples of the aorta were analyzed for specific components by chemical assays. The samples were weighed and frozen promptly after removal. For analysis, the weighed sections were thawed, the tissues were then minced and homogenized in an all-glass homogenizer containing 2:1 chloroform:methanol. The homogenizer was then rinsed twice with chloroform:methanol solution, followed by two rinses with 1% HCl. All rinses were pooled and the homogenate was allowed to extract for 2 hours. Carbon-14 (14C)-cholesterol, 5000 cpm, and coprostanol, 0.05 mg, were then added to each tube to aid in determining the percent recovery in the lipid analysis and as internal standards for the gas chromatographic analysis, respectively. The tubes containing the homogenate, chloroform:methanol, and HCl solutions were then centrifuged for 10 minutes at 1000 rpm. The organic layer was removed, dried, and redissolved in chloroform, which was then separated into three aliquots for cholesterol, triglyceride, and phospholipid analyses. The remaining aortic extract was dried at 50°C in a vacuum oven and weighed for fat-free dry weight determinations. It was then used for calculation of collagen values.

The quantitation of total, free, and esterified cholesterol was performed according to the method of Ishikawa, et al., as modified by Bates and Wissler. Briefly, the volume of the cholesterol peak relative to the nearby coprostanol peak is used to demonstrate the free cholesterol content of the sample, using gas liquid chromatography. After the free cholesterol content of a sample aliquot is determined, the entire sample is saponified to remove the fatty acid from the cholesterol esters in the sample. Chromatographic determination on this saponified sample provides quantitation of the total cholesterol content in the original cholesterol sample. The cholesterol ester content is determined by subtraction of the free cholesterol content from the saponified determination.

Triglycerides were separated by thin-layer chromatography using a developing system of petroleum ether, ethyl ether, and acetic acid in a 75:25:1 ratio. The triglyceride content was then quantitated according to the "charring" method of Kritchevsky, et al. Phospholipids were determined by the method of Bartlett.

Aortic collagen levels were determined by the hydroxyproline assay of Neuman and Logan as modified by Martin and Axelrod. Collagen was isolated from dried-delipidated aortic samples by extraction in 0.1 N NaOH at 98°C for 50 minutes. The insoluble residue was washed once with 0.1 N NaOH and twice with distilled water. The 0.1 N NaOH and distilled water extracts and washings were pooled in Teflon-capped tubes and adjusted to 6.0 N HCl. The collagen extract was hydrolyzed under air in a glycerol bath at 105°C for 48 hours, and 1-μl aliquots were then dried under vacuum with phosphorus pentoxide and sodium hydroxide. The dried hydrolysates were resuspended in distilled water, and the hydroxyproline content was determined with appropriate standards and blanks.

Micromorphometry

To evaluate lesions microscopically, we have developed methods for quantitating components at the light microscopic level from histological preparations. To quantitate the cellular and extracellular components of lesion sections we used a Hewlett-Packard System 45 digitizer plate-computer and microprojector combination. This consists of a projector and a reflecting mirror on adjustable mounts designed to provide a continuous range of magnification with minimal planar distortion of the projected images onto the digitizer plate. Light microscopic preparations of full circumferential sections are projected onto the digitizer plate, and contours are traced to evaluate lesion size, shape, and degree of luminal obstruction, as well as major lesion components such as necrotic zones, fibrous caps, calcifications, and fibrosis. The programs are designed to accelerate measurements and automate data recording and retrieval. Applied to arteries, these methods permit estimates of changes in relative volume and location of component structures by allowing selection of areas for study, such as lesions in the media and adventitia. With this equipment, quantitative determinations of lesion size and composition were performed on each animal.

Catecholamine Determinations

Catecholamine levels were measured in the myo-
cardium. Immediately following removal of the heart, biopsies were taken from the following places: the outflow area of the right ventricle, the anterior right ventricle, the posterior right ventricle, the lateral left ventricle, and the posterior left ventricle. Tissue was dissected, blotted dry, and quickly weighed; 15- to 20-μg fragments were frozen in liquid nitrogen and stored at −80°C until homogenized. At that time the frozen tissue was placed in a polypropylene tube containing 0.5 ml of a cold mixture of 0.1 M perchloric acid, 5 mM disodium ethylenediaminetetraacetic acid (EDTA), and 0.5 mM sodium metabisulfite solution containing 110 ng of dihydroxybenzylamine, which served as an internal standard to control for losses during processing. The tissue was disrupted first by a motor-driven epoxy pestle (500 rpm) until an apparently homogeneous suspension was obtained, and then by sonication for 15 seconds at medium power (Branson Sonifier 185 with a microtip). The homogenate was centrifuged at 4°C for 10 minutes at 37,700 G. The supernatant was removed, neutralized to pH 7.0 with Tris aminomethane base, and then extracted with A1203 and chromatographed by high-performance liquid chromatography with electrochemical detection as described by Mefford, et al., except that a Water's 5-μ radial pack C-18 column was used.

**Results**

All animals increased in weight steadily throughout the year that they were fed the atherogenic diet, although the increase was greatest in Group 1 (Table 3). Blood cholesterol levels increased rapidly from an initial basal average of 170 mg% to an average of 791 mg% in the group receiving a sympathectomy (Group 1), and to 913 mg% and 900 mg% in Groups 2 and 3, which received the atherogenic diet with and without propranolol, respectively. The average cholesterol level for each group is listed in Table 3. These values were determined by averaging the serum cholesterol levels for each group as measured bimonthly throughout the experiment. The propranolol group had a somewhat higher average cholesterol level while the sympathectomy group had a slightly lower average cholesterol level in comparison to the control monkeys. Serum high-density lipoprotein (HDL)-cholesterol was also measured and showed a decrease in Groups 2 and 3 during the year-long experiment, but no change was noted in the HDL-cholesterol level in Group 1 (Table 3). The serum triglyceride level increased markedly during the experimental period for Groups 2 and 3, but no difference was noted at terminal bleeding in serum triglycerides for Group 1 (Table 3). Physically the animals were healthy, alert, and vigorous throughout the experimental period. They showed no hematological or chemical abnormalities in their blood samples, except for the changes in blood lipid levels.

The gross measurements of the atherosclerosis present in the aortas and carotid and femoral arteries are outlined in Table 4. It is evident that the gross aortic atherosclerosis was consistently extensive in all three groups. However, the group with bilateral thoracic sympathectomy (Group 1) had less gross atherosclerotic involvement of the carotid arteries while the propranolol group (Group 2) had less gross involvement of the femoral arteries in comparison with the control group.

Using the micromorphometric methods outlined previously, quantitative measurements were made of the intimal lesions at standard regions in the major vessels, and the results are outlined in Tables 5 and 6. Clearly,

### Table 3

**Animal weights and serum lipid levels**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Weight (kg)</th>
<th>Average Cholesterol (mg%)</th>
<th>Triglycerides (mg%)</th>
<th>HDL-Cholesterol (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Terminal</td>
<td>Baseline</td>
<td>Terminal</td>
</tr>
<tr>
<td>1</td>
<td>9.9 ± 0.5</td>
<td>12.9 ± 0.8</td>
<td>641 ± 56</td>
<td>63 ± 7</td>
</tr>
<tr>
<td>2</td>
<td>10.0 ± 0.8</td>
<td>11.9 ± 1.1</td>
<td>848 ± 91</td>
<td>42 ± 10</td>
</tr>
<tr>
<td>3</td>
<td>9.9 ± 0.6</td>
<td>11.5 ± 0.7</td>
<td>735 ± 45</td>
<td>31 ± 6</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. HDL = high-density lipoprotein.

### Table 4

**Gross pathological vascular findings**

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Carotid Arteries</th>
<th>Aorta</th>
<th>Femoral Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lt</td>
<td>Rt</td>
<td>Thoracic</td>
</tr>
<tr>
<td>1</td>
<td>24 ± 6</td>
<td>24 ± 6</td>
<td>67 ± 10</td>
</tr>
<tr>
<td>2</td>
<td>31 ± 13</td>
<td>39 ± 13</td>
<td>67 ± 13</td>
</tr>
<tr>
<td>3</td>
<td>29 ± 9</td>
<td>31 ± 9</td>
<td>64 ± 11</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means, expressed as percent of atherosclerosis.
Sympathectomy and atherosclerosis

TABLE 5
Quantification of intimal lesions in the carotid arteries and thoracic aorta*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Carotid Arteries</th>
<th>Thoracic Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (sq mm)</td>
<td>Thickness (mm)</td>
</tr>
<tr>
<td>1</td>
<td>0.18 ± 0.03†</td>
<td>0.10 ± 0.01†</td>
</tr>
<tr>
<td>2</td>
<td>0.50 ± 0.15</td>
<td>0.22 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>1.13 ± 0.36</td>
<td>0.43 ± 0.12</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. Area = area of intimal plaque; thickness = maximum intimal thickness.
† Significance of difference: p < 0.05 compared to Groups 2 and 3.
‡ Significance of difference: p < 0.05 compared to Group 3.

the intimal area, thickness, and luminal stenosis of the carotid arteries in the sympathectomy group were markedly less than that found with the control group, in agreement with the gross observations (p < 0.05). A photomicrograph of a representative carotid artery lesion stained for lipid detection from an animal fed a cholesterol-rich butterfat and coconut oil diet and subjected to bilateral surgical sympathectomy is shown in Fig. 1. Figures 2 and 3 illustrate typical carotid artery lesions in monkeys fed the atherogenic diet in combination with propranolol (40 mg/12 hrs) or alone. The lesions in the carotid arteries of animals with bilateral sympathectomy were mildest (Fig. 1), while those in animals fed the atherogenic diet alone were most severe (Fig. 3). The carotid arteries from the propranolol-treated group demonstrated less disease than that seen in the control animals but somewhat more than in the sympathectomy group (Table 5). The intimal lesions in the thoracic and abdominal aorta (Tables 5 and 6) followed a pattern similar to that seen in the carotid artery lesions, although the differences were less marked; the abdominal aortic lesions showed the least difference in the three groups. Finally, the most severe intimal disease in the femoral arteries was seen in the group receiving propranolol (Table 6); the bilateral

TABLE 6
Quantification of intimal lesions in the abdominal aorta and femoral arteries*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Abdominal Aorta</th>
<th>Femoral Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (sq mm)</td>
<td>Thickness (mm)</td>
</tr>
<tr>
<td>1</td>
<td>0.30 ± 0.06†</td>
<td>0.12 ± 0.03†</td>
</tr>
<tr>
<td>2</td>
<td>0.72 ± 0.19</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>0.84 ± 0.17</td>
<td>0.29 ± 0.06</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. Area = area of intimal plaque; thickness = maximum intimal thickness.
† Significance of difference: p < 0.05 compared to Group 3.

thoracic sympathectomy group had involvement in the femoral arteries similar to that of the control group. The microscopic data from the coronary arteries (left anterior descending, left circumflex, and right circumflex arteries) are shown in Table 7. Although the amount of atherosclerotic disease was fairly minimal in all three groups, somewhat less luminal occlusion was seen in the propranolol-fed group.

The biochemical analysis of the thoracic and abdominal aortas from the three groups is outlined in Table 8. The cholesterol content was slightly higher in the
TABLE 7
Quantification of lesions in the coronary arteries*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Area (sq mm)</th>
<th>Thickness (mm)</th>
<th>Luminal Stenosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.08 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>5.4 ± 1.1</td>
</tr>
<tr>
<td>2</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>3.3 ± 0.51</td>
</tr>
<tr>
<td>3</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.01</td>
<td>5.9 ± 0.08</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. Area = area of intimal plaque; thickness = maximum intimal thickness.

The phospholipid and collagen contents were similar in all three groups for the abdominal aorta, although the thoracic aorta from the sympathectomy group contained somewhat more collagen (p < 0.05).

The norepinephrine levels measured in tissue from six distinct points of the hearts of these animals are outlined in Table 9. Clearly, an approximately 50% reduction of norepinephrine was found at each point for the sympathectomy group while only a slight reduction of norepinephrine was noted in the propranolol-treated group (most marked at the anterior and posterior left ventricle).

The mean arterial blood pressure (MABP) and heart rate were measured only once and that was just prior to autopsy. The results are summarized in Table 10. There was at this time a marked reduction in MABP in the sympathectomy group but the value for the propranolol group was identical to that of the controls. No difference in heart rate was noted among the three groups. Clearly, many more determinations in unanesthetized animals would have to be obtained in order to develop a clear understanding of the influence of these variables on the development of arterial lesions in this study. Nevertheless, it is obvious that the marked reduction in MABP observed in the sympathectomy group was not accompanied by a comparable effect on the aortic disease.

TABLE 8
Results of biochemical analysis of the thoracic and abdominal aorta*

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Total Cholesterol</th>
<th>Free Cholesterol</th>
<th>Cholesterol Ester</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>thoracic aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13.2 ± 2.8</td>
<td>7.9 ± 1.3</td>
<td>5.3 ± 1.6</td>
<td>2.6 ± 0.07</td>
<td>6.4 ± 0.6</td>
<td>11.0 ± 0.6†</td>
</tr>
<tr>
<td>2</td>
<td>27.1 ± 7.5</td>
<td>15.3 ± 4.4</td>
<td>11.8 ± 3.3</td>
<td>1.0 ± 0.4</td>
<td>5.3 ± 0.5</td>
<td>10.2 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>16.8 ± 3.4</td>
<td>9.0 ± 1.4</td>
<td>7.8 ± 2.2</td>
<td>2.4 ± 0.6</td>
<td>5.5 ± 0.4</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>abdominal aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.2 ± 1.2</td>
<td>4.1 ± 0.8</td>
<td>3.1 ± 0.6</td>
<td>1.8 ± 0.5</td>
<td>6.6 ± 0.6</td>
<td>16.9 ± 0.8</td>
</tr>
<tr>
<td>2</td>
<td>21.8 ± 5.7</td>
<td>13.1 ± 3.1</td>
<td>8.8 ± 2.9</td>
<td>1.2 ± 0.8</td>
<td>5.2 ± 0.5</td>
<td>11.9 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>10.6 ± 3.2</td>
<td>5.6 ± 1.5</td>
<td>5.0 ± 1.7</td>
<td>0.9 ± 0.4</td>
<td>5.2 ± 0.4</td>
<td>14.2 ± 1.1</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means (µg/mg wet weight).
† Significance of difference: p < 0.05 compared to Group 3.

Discussion

It is clear from these data that there was a marked reduction in the progression of atherosclerosis in the carotid arteries of monkeys who had undergone a bilateral thoracic sympathectomy prior to being fed an atherogenic diet for 1 year. There was also a moderate reduction in the progression of atherosclerosis in the thoracic aortas of these animals with a smaller reduction in the abdominal aorta. Although this group had a sustained cholesterol level somewhat less than the control group (641 vs. 735 mg%), the amount of atherosclerosis in the femoral arteries was slightly more than that in the sympathectomy group, suggesting that a protective effect in the neck and chest arteries did result from the bilateral thoracic sympathectomy, with the...
femoral arteries still receiving sympathetic input from the lumbar chain. Propranolol at a dose of 40 mg/12 hrs also seemed to offer a protective effect against the progression of atherosclerosis in the carotid arteries and the thoracic aorta, although the degree of protection was less than that offered by the surgical sympathectomy. It is interesting to note that propranolol was not effective at this dose in controlling atherosclerosis in either the abdominal aorta or the femoral arteries, although a very mild beneficial effect was seen in the left circumflex coronary artery.

The cholesterol content of the vessels seemed to be a reflection of the serum cholesterol level; the most deposits appeared in the propranolol group, which had the highest overall average cholesterol level (848 mg%). There was a small increase in triglyceride deposition in both the thoracic and abdominal aorta of the sympathectomy group; the significance of this is unclear, especially in light of the low serum triglyceride level found in this group at the termination of the experiment. Similar findings were described by Fronek, et al., in rabbits and rats subjected to a chemical sympathectomy with 6-hydroxydopamine.

It is important to note that the norepinephrine levels were only reduced by approximately 50% in the heart muscle of the monkeys receiving a bilateral thoracic sympathectomy, indicating only partial reduction of catecholamine input to the major vessels from this procedure. The MABP, measured only once at the conclusion of the experiment, appeared to be significantly reduced in the monkeys which had been treated with a surgical sympathectomy; at that time the animals were all tranquilized, and the average heart rates were similar in all of the groups. Although propranolol did not seem to affect heart rate or blood pressure of animals after 1 year on the drug, a transient reduction in blood pressure has been observed in conscious rabbits fed comparable doses of propranolol.37

The apparent protective mechanism of \( \beta \)-adrenergic blockade in the progression of atherosclerosis suggested by this study remains unclear. It has been well established that \( \beta \)-adrenergic blockade in the primate causes a decrease in cardiac output.28 The animal maintains an unaltered arterial pressure by active vasoconstriction of the various vascular beds, with the brain affected least. Transient decreases in blood pressure lasting several months could be significant in retarding atherosclerosis over a period of 1 year. Furthermore, \( \beta \)-adrenergic blockade may prevent transient elevations in blood pressure in response to stress, which could be significant in reducing atherosclerosis.14 Vasodilation should result from surgical sympathectomy and this, combined with a lowering of systemic blood pressure, may be a factor in retarding atherosclerosis. In addition, investigators have proposed that centrally mediated autonomic responses to stress may play a role in atherosclerosis.15 It has also been suggested that circulating catecholamines may foster arterial lesions directly as well as through influences on pathogenic factors such as platelet aggregation.13

In summary, sympathectomy and propranolol treatment of monkeys resulted in smaller atherosclerotic lesions, which were more lipid-rich in the propranolol-fed group. Sympathetic blockade appears to reduce the development of atherosclerotic lesions, especially in selected arterial beds such as the carotid arteries. Further studies to evaluate potential mechanisms by which sympathetic innervation controls atherogenesis are in progress.

References

cholesterol accumulation in monkey aortic medial cells. Biochim Biophys Acta 450:78-88, 1976

Address reprint requests to: Terry Lichtor, M.D., Ph.D., Section of Neurological Surgery, Hospital Box 405, The University of Chicago Medical Center, 5841 South Maryland Avenue, Chicago, Illinois 60637.

T. Lichtor, et al.

J. Neurosurg. / Volume 67 / December, 1987