Experimental intracranial aneurysms in rats

A gross and microscopic study

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A number of animal studies have been performed in which arterial aneurysms were produced, little is known concerning the nature of such aneurysms in cerebral vessels. Very recently, Hashimoto, et al., reported the successful production of saccular cerebral aneurysms in rats treated with β-aminopropionitrile fumarate (BAPN). In their experiments, hypertension was also induced by the deoxycorticosterone acetate and salt (DOCA-salt) method, and ligation of a single common carotid artery was performed.

In the present report, the experimental findings of Hashimoto, et al., were confirmed and extended to include the following: BAPN food was given in lower concentrations to ascertain whether death might be delayed and the incidence of aneurysms increased. In addition, hypertension was induced by the Goldblatt method rather than with DOCA-salt to determine whether the toxic effects of these substances were essential for the genesis of intracranial aneurysms. The influence of estrogen on the production of aneurysms was also evaluated because estradiol experimentally reduces the level of both collagen and elastin in castrated male rats, and because reports indicate that women have a greater incidence of cerebral aneurysms than men. Histological studies were performed to better define the nature of these aneurysms.

Materials and Methods

Experimental Groups

Eighty adult male Sprague-Dawley rats weighing approximately 360 gm were separated into seven groups as shown in Table 1. Adult male rats were selected because Hashimoto, et al., reported that the incidence of aneurysms induced experimentally was highest in this sex. The groups studied were as follows.

Group I animals were subjected to the same conditions as those of Hashimoto, et al., Right nephrectomy and left common carotid artery ligation were performed under intraperitoneal pentobarbital anesthesia (35 to 40 mg/kg), followed by semi-weekly subcutaneous injections of DOCA (2.5 mg/100 mg of body weight) and ingestion ad libitum of drinking water as a 1% NaCl solution, beginning 1 week after surgery. Rat food pellets containing 0.12% concentration of BAPN (0.12% BAPN food)* was offered ad libitum beginning 2 weeks after surgery.

In Group II, 22 rats were subjected to the same conditions as those in Group I, except that the BAPN concentration in the food was reduced to 0.1%.

* BAPN food was prepared by Teklad Test Diets, Madison, Wisconsin.
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### TABLE 1
Procedures for the induction of cerebral saccular aneurysms in adult male rats

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of Rats</th>
<th>BAPN in Food (%)</th>
<th>Deoxycorticosterone*</th>
<th>1% NaCl in Water</th>
<th>Carotid Ligation</th>
<th>Kidney Removed</th>
<th>Other Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>19</td>
<td>0.12</td>
<td>2.5 mg/100 gm</td>
<td>yes</td>
<td>left</td>
<td>right</td>
<td>none</td>
</tr>
<tr>
<td>II</td>
<td>22</td>
<td>0.1</td>
<td>2.5 mg/100 gm</td>
<td>yes</td>
<td>left</td>
<td>right</td>
<td>none</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>0.1</td>
<td>none</td>
<td>left</td>
<td>right</td>
<td>none</td>
<td>renal artery stenosis</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>0.1</td>
<td>none</td>
<td>left</td>
<td>right</td>
<td>none</td>
<td>renal artery stenosis, castration, estradiol 50 μg/wk</td>
</tr>
<tr>
<td><strong>Control groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>none</td>
<td>2.5 mg/100 gm</td>
<td>yes</td>
<td>left</td>
<td>right</td>
<td>none</td>
</tr>
<tr>
<td>VI</td>
<td>5</td>
<td>0.1</td>
<td>none</td>
<td>left</td>
<td>right</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>VII</td>
<td>5</td>
<td>0.12</td>
<td>none</td>
<td>left</td>
<td>right</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

*Deoxycorticosterone given subcutaneously semiweekly.

Group III comprised 15 rats. In this group, hypertension was induced by the Goldblatt technique, rather than by DOCA-salt. The left renal artery was constricted with a silver clip devised by Brooks, et al.3 and a right nephrectomy and left common carotid artery ligation were performed. A BAPN concentration in the food (0.1%) was started 1 week after surgery.

Group IV contained nine rats. This group of rats was feminized by bilateral orchiectomy, then injected subcutaneously with estradiol (50 μg/week), started during the 1st postoperative week. Otherwise, the animals were treated in the same way as those of Group III.

Groups V, VI, and VII served as controls, and consisted of five rats each. Hypertension was induced with DOCA-salt, and a left common carotid artery ligation was performed in Group V. Only 0.1% and 0.12% BAPN food was given to animals in Groups VI and VII, respectively.

**Experimental Protocol**

The blood pressure of the rats was measured without anesthesia once before and weekly after surgery by the tail plethysmographic method, using a pulse transducer instrument attached to a recorder.† The mean blood pressure was computed from the recorded systolic and diastolic pressures. Angiography was performed in 21 rats, 8 to 10 weeks after the start of the experiment, by direct puncture of the exposed right common carotid artery.

Twenty-one rats were sacrificed by intraperitoneal injections of 50 to 60 mg of sodium pentobarbital. The brain was removed and examined with the Zeiss operating microscope. The thoracic and peritoneal cavities were examined macroscopically. The cerebral aneurysms produced in this experiment were photographed, sectioned, embedded, and stained with hematoxylin and eosin, azocarmine (for fibrin and collagen), and the Verhoeff-van Gieson (elastic tissue) methods.

**Series of Dogs**

In an attempt to produce aneurysms in a larger species, we included in the study 10 adult mongrel dogs weighing between 9 and 15 kg (average 12.7 kg). These animals underwent unilateral nephrectomy and ligation of the right carotid and right vertebral arteries under anesthesia. They were then given DOCA (10 mg/kg daily), supplied with drinking water with a 1% NaCl solution, and fed *ad libitum* food pellets containing 0.05% BAPN, starting 2 weeks after surgery. All of these dogs manifested anorexia and marked motor disturbances within 1 week of receiving this food. These motor deficits primarily consisted of an inability to extend the extremities. Despite cessation of the BAPN feeding on the 9th day, eight of 10 dogs died within 3 weeks. In one of these eight dogs, subarachnoid hemorrhage was found at autopsy, but there were no gross vascular abnormalities nor aneurysms in any of these animals. Because of these findings this study was discontinued.

**Results**

**Size and Location of Aneurysms**

The primary findings are summarized in Tables 2 and 3. Aneurysms located at the base of the brain were identified with the operating microscope and grossly in or near the circle of Willis in nine rats. Four of these were in Group I, three in Group III, and two in Group IV (Table 2). None of the aneurysms had ruptured. The most common site was the terminal portion of the A1 segment of the anterior cerebral artery near the...
TABLE 2

General results obtained in the various groups of rats studied

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Total Rats</th>
<th>Rats with Saccular Aneurysms</th>
<th>Cause of Death and Duration of BAPN Feeding</th>
<th>Other Gross Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No. Percent</td>
<td>Intracran Hem</td>
<td>Periton Hem</td>
</tr>
<tr>
<td>I</td>
<td>19</td>
<td>4 21</td>
<td>1 6 (38 ± 2.5)</td>
<td>4 (31, 47)</td>
</tr>
<tr>
<td>II</td>
<td>22</td>
<td>0 11</td>
<td>1 16 (38 ± 1.4)</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td>3 20</td>
<td>14 93 (51 ± 3.2)</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>2 22</td>
<td>5 6 (52, 74)</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>0 5 100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mean blood pressure greater than 150 mm Hg.
†Values in parentheses indicate duration in days (if more than two, mean ± S.E.M.) from onset of BAPN feeding to death. Intracran Hem = intracranial hemorrhage; Periton Hem = peritoneal hemorrhage; Thor Hem = thoracic hemorrhage.
++Days from surgery to death.

anterior cerebral trunk (which probably corresponds in location to the anterior communicating artery of man), just above the optic chiasm (Table 3). Two rats in Group I (Rats AN1 and AN2) and two in Group IV (Rats AN8 and AN9) had small aneurysms (0.1 to 0.2 mm in diameter) in this location. One of these four rats (Rat AN8) had two aneurysms here (Fig. 1). All of these aneurysms were on the right side. The next most common location was the proximal part of the left posterior cerebral artery in the interpeduncular cistern. Three aneurysms were found in this location. Aneurysms of two rats in Group I (Rats AN3 and

TABLE 3

Experimental details in nine rats with cerebral saccular aneurysms*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Group No.</th>
<th>Site of Aneurysm</th>
<th>BAPN in Food (%)</th>
<th>Cause of Hypertension</th>
<th>Last BP (mm Hg)</th>
<th>Cause of Death</th>
<th>Days From BAPN Feeding to Death</th>
<th>Other Notable Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN1</td>
<td>I</td>
<td>rt ACA</td>
<td>0.12</td>
<td>DOCA-salt</td>
<td>170</td>
<td>postangiographic</td>
<td>44 none</td>
<td>none</td>
</tr>
<tr>
<td>AN2</td>
<td>I</td>
<td>rt ACA</td>
<td>0.12</td>
<td>DOCA-salt</td>
<td>150</td>
<td>undetermined</td>
<td>62 none</td>
<td>none</td>
</tr>
<tr>
<td>AN3</td>
<td>I</td>
<td>lt PCA</td>
<td>0.12</td>
<td>DOCA-salt</td>
<td>160</td>
<td>sacrificed</td>
<td>31 rt occipital hemorrhage, neurological signs</td>
<td>mesenteric artery nodules</td>
</tr>
<tr>
<td>AN4</td>
<td>I</td>
<td>lt PCA</td>
<td>0.12</td>
<td>DOCA-salt</td>
<td>180</td>
<td>peritoneal hemorrhage</td>
<td>47 none</td>
<td></td>
</tr>
<tr>
<td>AN5</td>
<td>III</td>
<td>rt ACA</td>
<td>0.1</td>
<td>Goldblatt</td>
<td>130</td>
<td>sacrificed</td>
<td>103 none</td>
<td></td>
</tr>
<tr>
<td>AN6</td>
<td>III</td>
<td>rt ICA bifurcation</td>
<td>0.1</td>
<td>Goldblatt</td>
<td>160</td>
<td>peritoneal hemorrhage</td>
<td>54 none</td>
<td></td>
</tr>
<tr>
<td>AN7</td>
<td>IV</td>
<td>lt PCA</td>
<td>0.1</td>
<td>Goldblatt</td>
<td>150</td>
<td>sacrificed</td>
<td>103 none</td>
<td></td>
</tr>
<tr>
<td>AN8</td>
<td>IV</td>
<td>rt ACA (multiple)</td>
<td>0.1</td>
<td>Goldblatt</td>
<td>130</td>
<td>sacrificed</td>
<td>111 none</td>
<td></td>
</tr>
<tr>
<td>AN9</td>
<td>IV</td>
<td>rt ACA</td>
<td>0.1</td>
<td>Goldblatt</td>
<td>140</td>
<td>sacrificed</td>
<td>111 none</td>
<td></td>
</tr>
</tbody>
</table>

*ACA = anterior cerebral artery; PCA = posterior cerebral artery; ICA = internal carotid artery; DOCA = deoxycorticosterone acetate; and BP = blood pressure.
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AN4) were relatively small, being 0.3 and 0.4 mm in diameter, respectively. An aneurysm in one rat of Group III (Rat AN7) was relatively large, measuring 0.9 mm in diameter. Two other rats had aneurysms: one measured 0.4 mm in diameter at the bifurcation of the right internal carotid artery (Rat AN6), and the other was present at the origin of an arterial branch of the proximal portion of the A1 segment of the right anterior cerebral artery, and measured 0.6 mm (Rat AN5) (Fig. 2). None of the intracranial aneurysms, however, were detected arteriographically.

The only other location of intracranial vascular lesions was in the right occipital lobe, found in 10 rats. In seven of these, only a single small cortical hemorrhage was encountered. In two cases, the hemorrhage was both intracortical and subarachnoid, and may have been the cause of death, but no aneurysms were found at the base of the brain. In one rat, an area of old encephalomalacia was noted.

The time interval between the initial BAPN feeding and recognition of an aneurysm ranged from 31 to 111 days. The second largest aneurysm in this series (in Rat AN6) was found 54 days after the start of BAPN feeding. No correlation was found between the duration of the experimental conditions and the size of the aneurysm.

Incidence and Degree of Hypertension

There was no absolute relationship between the occurrence of aneurysms and the degree of hypertension. The average blood pressure prior to surgery was 97.5 mm Hg (range, 90 to 125 mm Hg). In this study, a mean blood pressure recording higher than 150 mm Hg was arbitrarily regarded as systemic hypertension. The pressures in these rats ranged from 150 to 180 mm Hg (average, 161.5 mm Hg). Six rats (Rats AN1, AN2, AN3, AN4, AN6, and AN7) with saccular aneurysms had hypertension, but three rats (Rats AN5, AN8, and AN9), which had smaller anterior cerebral artery aneurysms, were normotensive by definition. The blood pressure in these three animals terminally was higher than 120 mm Hg. Hypertension was induced most successfully in Group III animals (93%) with the Goldblatt method. About 74% of the Group I rats, treated with DOCA-salt, manifested hypertension, while the rats in Groups II and IV had an incidence of hypertension of about 50% (Table 2). All five rats of control Group V, treated with DOCA-salt, developed hypertension. Hypertension was first observed during the 3rd week after surgery in a few animals. Thereafter, progressively more animals became hypertensive. No significant difference in time of onset or degree of hypertension was seen between the animals subjected to the different procedures. However, death tended to occur earliest (37 days compared to 51 days) in Group I and II animals, which received DOCA-salt (Table 2).

Other Findings

Extracranial vascular lesions were also noted. Intrathoracic and intra-abdominal vascular changes and hemorrhage were found, and these evidently were the most common causes of death among the experimental groups. Intrathoracic hemorrhage was seen only in Group I rats, as a result of dissecting aneurysms of the aorta. Gross nodular aneurysmal changes in the mesenteric arteries were found in five rats without intra-abdominal hemorrhage. Peritoneal hemorrhage was the most common cause of death in rats treated with 0.1% BAPN food, except for those in Group IV in which feminization was induced. Enlarged

Fig. 1. Rat AN8. Two aneurysms are shown on the A1 segment of the right anterior cerebral artery (arrows). The more proximal aneurysm is clearly located at a branching site. The larger distal aneurysm is also at a branching site, although the branch is not apparent in the photograph. The optic chiasm and nerves at the bottom of the picture had been cut.

Fig. 2. Rat AN5. A large aneurysm on the right anterior cerebral artery can be seen arising proximally at the origin of a large arterial branch (arrow).
hemorrhagic lymph nodes were always associated with peritoneal hemorrhage.

Only one rat (Rat AN3) showed neurological signs and emaciation, and therefore was sacrificed. Four other rats that were sacrificed (Rats AN5, AN7, AN8, and AN9) appeared healthy at the time of death. Two rats (Rats AN4 and AN6) died of peritoneal hemorrhage, and one rat (Rat AN1) died following angiography. The cause of death of one rat (Rat AN2) was not determined. Marked deformity of the thoracic spine and paraplegia were observed in three rats, all of which had received 0.12% BAPN food.

**Histology**

The saccular aneurysms evident at the base of the brain had many histological features in common, and therefore will be described together (Fig. 3). The wall of the aneurysmal sac was composed primarily of connective tissue which varied in thickness. The internal elastic membrane was interrupted at the origin of the aneurysm, was rarely fragmented, and was absent from the wall of the aneurysm (Fig. 3 left). All aneurysms showed subendothelial deposition of smudgy eosinophilic material, portions of which stained bright red with azocarmine techniques, suggesting its fibrinoid character (Fig. 3 right). The saccular wall of some aneurysms appeared thickened, due in part to infiltration with chronic inflammatory cells and extravasation of red blood cells. Poorly organized thrombotic material was present in one aneurysm. Relatively modest numbers of chronic inflammatory cells frequently infiltrated the wall of the aneurysm, extending in some cases into the adjacent and overlying leptomeninges.

The small cortical and leptomeningeal vessels of the right occipital lobe showed striking changes (Fig. 4). These vessels were enlarged, their walls were thickened, and abundant fibrinoid material was present in many of them. Some were frankly fibrotic. A variable chronic inflammatory cell infiltrate and fibrosis were encountered in the leptomeninges and cortex in one case.

The nodular lesions of the mesenteric artery walls were composed of chronic inflammatory cells and fibroblasts, confined mostly to irregular thickenings of the adventitia and media. One nodule contained epithelioid cells, but no giant cells were encountered. The intima showed focal hyperplasia in these lesions, and scanty subintimal fibrinoid material was seen. Only remnants of the elastica interna remained. There was also chronic inflammation in perivascular mesenteric tissue.

**Discussion**

Previous efforts to produce experimental changes simulating intracranial berry aneurysms have been discarded for technical and other reasons. Recently, however, Hashimoto and his associates successfully produced experimental saccular aneurysms by a combination of angiolathyrism, hemodynamic stress (unilateral carotid artery ligation), and induced hypertension. The present study confirms their observations; moreover, it defines these aneurysms histologically, and proves that the administration of DOCA-salt is not a requisite for producing this phenomenon. Although the highest in-
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Fig. 4. Photomicrographs of a section from the right occipital lobe of a rat from Group I. Five vessels, some enlarged to varying degrees, have thickened walls due to deposition of acellular fibrinoid material. Fresh cortical hemorrhage is illustrated in the right lower portion of the photograph. H & E, × 74.

The incidence of aneurysms occurred in rats treated with DOCA-salt, the much earlier death of these animals, as anticipated, would make them less useful for long-term manipulation and study. Despite one report of aneurysm-like changes following common carotid artery ligation alone, we found no microscopic vascular changes in any control animal, or with BAPN feeding alone. The present findings suggest that a 0.1% or 0.12% BAPN diet, together with Goldblatt hypertension and carotid artery ligation, is the best method to induce intracranial saccular aneurysms in rats. The value of feminization requires further study; however, one should note that the animals thus treated lived longer and had a high incidence of aneurysms. Perhaps estradiol plays a determinant role.

Aneurysms produced by this method have many features in common with human berry aneurysms. Like berry aneurysms, they occur on the vessels of the circle of Willis, not infrequently at branching sites, and they vary in size. They are composed of connective tissue, and are essentially devoid of smooth muscle and elastica. They differ, however, from the common saccular aneurysm in the fact they invariably show fibrinoid necrosis and chronic inflammatory cells. The aneurysms previously reported by Hashimoto, et al., were not examined histologically, but it is likely that their changes were similar to those recorded here. In three of five of their rats, the aneurysms were located on arteries at the base of the brain. One of these had multiple small aneurysms at the “anterior cerebral-anterior communicating complex” which is the most common site of the aneurysms induced in our study. It is worthwhile to emphasize that changes were also encountered in vessels other than those of the circle of Willis. Smaller leptomeningeal and cortical vessels were encountered that also showed fibrinoid necrosis and chronic inflammatory cells, but only in the right cerebral hemisphere; these cases were associated with subarachnoid and/or intracranial hemorrhage. The finding that these intracranial vascular lesions occurred only in branches opposite the occluded left common carotid artery indicates that hemodynamic factors played a determinant role in the development of the vasculopathies observed.

Vasculopathy was also present in the mesenteric vessels; this is substantially different from previous descriptions of aneurysms in the aorta, and is probably identical to those observed grossly and termed “periarteritis nodosa” by others. The salient histological features were marked, chronic, occasionally granulomatous, inflammatory reaction and fibrinoid necrosis. These histological changes suggest that the etiology of the vasculopathy is, at least in part, toxic, leading to the chronic inflammatory reaction. The role of hypertension in the evolution of some of the changes, such as fibrinoid necrosis and fibrosis, must also be considered; however, three of nine aneurysms in this study were found in so-called normotensive rats. Nevertheless, it is reasonable to suspect that the elevated intra-arterial pressure in the hypertensive animals may have further weakened the arterial wall, leading to the enlargement of the aneurysm.

The success of lathyrism in inducing aneurysms requires an animal susceptible to angiolithism, and the avoidance of hemo- and osteolathyrism. All dogs studied showed early neurolathyrism, and died within a few weeks without gross vasculopathy. This undoubtedly is a species-related peculiarity, and it is yet to be determined whether additional modifications in technique will lead to successful induction of intracranial aneurysms in dogs. For example, BAPN may be withheld. This could result in a longer course, reduction of the inflammation, and a condition even more similar to the congenital aneurysms. Nevertheless, the intracranial aneurysms induced in rats have marked gross and microscopic similarities to human berry aneurysms, and may prove to be a useful model for the study of the development, pathophysiology, and therapy of this disorder.

Addendum

Since this paper was submitted for review, an article by Hashimoto and co-workers has appeared (Hashimoto N, Handa H, Hazama F: Experimentally induced cerebral aneurysms in rats. Part III: Pathology. Surg Neurol 11:299–304, 1979) in which the aneurysms produced by their original method were studied histologically. Their microscopic findings agree basically with ours except for the infiltration of chronic inflammatory cells into the aneurysm.
Acknowledgments

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References


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