The Effect of Hypophysectomy on Experimental Endothelial Cell Regrowth and Intimal Thickening in the Rat

MICHAEL A. BETTMANN, MICHAEL B. STEMERMAN, AND BERNARD J. RANSIL

SUMMARY We studied the influence of hypophysectomy on endothelial cell regrowth and intimal thickening following aortic endothelial removal. Modification of this influence by replacement doses of glucocorticoids, mineralocorticoids, thyroxin, and growth hormone also was investigated. Young adult male rats were used, and endothelial removal was achieved by the balloon catheter method. Percent endothelial regrowth and percent intimal thickening were quantified 1 or 2 weeks after injury (3 or 4 weeks after hypophysectomy). One week after injury, endothelial regrowth was 64% in hypophysectomized rats and only 54% in controls. At 2 weeks, values were 90% and 79% respectively. Intimal thickening (percent of wall thickness due to intima) at 1 week was 11% in hypophysectomized and 14% in control rats. At 2 weeks, values were 13% and 22%, respectively. All differences between hypophysectomized and control rats at 2 weeks were statistically significant. Neither endothelial regrowth nor intimal thickening in hypophysectomized rats was altered by hormone replacement. Comparison of areas in which endothelium had not regrown suggested that hypophysectomy had a direct effect on intimal smooth muscle cell proliferation. Thus, hypophysectomy suppresses intimal thickening and accelerates endothelial regrowth after wall injury. Neither effect depends on certain known hormones, and these effects are to some extent independent of one another. These findings are relevant to recent work on growth factors and atherogenesis.


DIFFUSE intimal thickening is considered by many to be a precursor of atherosclerotic plaque. It appears to depend mainly on two vascular cells, endothelial (EC) and smooth muscle (SMC). SMC's are present sparsely in the intact intima but are the major cellular component of both the media and the fibromusculoelastic plaque (Ross and Glomset, 1973; Sapa et al., 1975). They are known to synthesize elastin, collagen, and glycosaminoglycans (McCullagh and Balian, 1975; Wight and Ross, 1975; Narayan et al., 1976). Intimal thickening after wall injury, as well as accumulation of connective tissue and lipid in the vessel wall, has been shown to be influenced by endothelial cell coverage (Schoeffl and French, 1968; Stemerman and Ross, 1972; Ross and Glomset, 1976). Furthermore, intact endothelium is non-thrombogenic, and removal of the endothelium allows platelet thrombi to form on the subendothelial surface (Ross and Glomset, 1976). During formation of these thrombi, platelets undergo aggregation and the release reaction with secretion of intracellular materials (Witte et al., 1978; Ross and Vogel, 1978). Among these is a factor(s) which can induce migration and proliferation of medial SMC, thereby promoting intimal thickening (Antoniades et al., 1975; Friedman et al., 1977; Antoniades and Scher, 1977; Ross and Vogel, 1978).

The pituitary gland influences SMC proliferation: ablation of the pituitary leads to suppression of intimal thickening after endothelial removal (Tiell et al., 1978). The intent of this study was to determine whether certain endocrine hormones were able to restore SMC proliferation in a hypophysectomized animal, and to evaluate the characteristics of endothelial cell growth after removal of the gland.

Methods

Animal Model

The experimental animals were 400 ± 25-g young adult, male Sprague-Dawley rats (Zivic-Miller Laboratories). Hypophysectomy was performed by the supplier by trans-sphenoidal suction curetage. Litter-matched rats were used as controls. Rats were housed three per cage, at 25°-30°C. They were fed standard rat chow (Basal Diet; Ralston Purina Company). Liquid was given ad libitum: tap water to control rats and a modified salt solution to hypophysectomized rats. The salt solution consisted of 8.12 g NaCl, 0.232 g KCl, 0.128 g MgCl₂, and 0.264 g CaCl₂ in 4 liters of distilled water. Daily weights, as well as random blood pressures, were recorded for all animals.
Experimental Groups

Rats were placed in one of six groups (Table 1) with 12-15 animals per group. Groups I-IV consisted of hypophysectomized animals; groups V and VI were litter-matched controls. Hormone supplements were administered in peanut oil vehicle by once-daily subcutaneous injections in doses which previously have been shown to give at least replacement levels (Hess and Riegle, 1972; Denckla, 1973; Denckla, 1974). Group I rats received 20 μg/kg thyroxine, 0.3 mg/kg corticosterone, and 10 μg/kg deoxytocosterone acetate (DOCA; Sigma Chemical Co.) in 0.1 ml peanut oil. Group II received the above three hormones plus 0.4 IU/kg bovine growth hormone (National Pituitary Agency); group III received daily injections of 0.1 ml of the peanut oil vehicle alone, and group IV received no supplementations or injections. In the control groups, animals in group V received no supplementations or injections and those in group VI received daily 0.1 ml peanut oil injections. Injectisons of supplements in peanut oil or of peanut oil alone were begun on the 2nd day after hypophysectomy. Balloon de-endothelialization was carried out on the 15th day after hypophysectomy, and animals were killed 7 or 14 days later. Several control animals were killed immediately after de-endothelialization and others were sham-operated (aorta opened but not balloononed) and killed 2 weeks subsequently.

Endothelial Removal and Tissue Preparation

Aortic de-endothelialization was performed by the balloon catheter method (Baumgartner, 1963; Stemerman, 1973), using a #2 French Fogarty embolectomy catheter (Edwards Laboratories). Thirty minutes prior to death 2 ml of Evans Blue (T 1824, 4.52 mg/ml, Harvey Laboratories, Inc.) were injected intravenously. The rats then were killed by cardiac puncture and perfusion-fixation with 2% gluteraldehyde in 0.1 M cacodylate at pH 7.4 and 7% sucrose-0.1 M cacodylate at pH 7.4 and room temperature, at a perfusion pressure of 100 mm Hg for 15 minutes (Stemerman et al., 1977). The entire aorta was then removed intact and gently cleaned of adventitia. Kidneys and testes were removed and weighed. The aorta was fixed for an additional 3 hours, then stored for 12 hours in 7% sucrose-0.1 M cacodylate at pH 7.4. Each aorta was opened longitudinally, pinned-out en face, measured for length and width, and photographed at 6x. The transparency subsequently was used for quantification of EC regrowth (vide infra). Cross-sections were cut from: (1) between the 2nd and 3rd intercostal arteries, (2) between the 8th and 9th intercostal arteries, (3) just below the celiac axis, and (4) just above the aortic bifurcation. Each section was post-fixed in 2% osmium tetroxide for 1 hour, stained en bloc with 1% uranylacetate for 30 minutes, dehydrated through ethanol, and individually flat-embedded in Epon 812. The Epon blocks were trimmed and 1-μm-thick sections were sliced on an ultramicrotome (MT-2, Sorvall Division of Dupont). The thick sections were stained by toluidine blue and basic fuchsin, cover-slipped, and evaluated by light microscopy both qualitatively and quantitatively for intimal and medial thickness. Selected blocks were used to make thin sections for transmission electron microscopic evaluation.

Stereological Evaluation

For quantification of endothelial cell regrowth (ECR), a Dokumetere #1 (aus Jena) was used to project the photographic transparencies of the aorta en face at 17.5× onto a cutom-made coherent multipurpose lattice (Weibel, 1973) containing 8906 tests points spaced 0.5 mm apart. The numbers of points falling on blue, dark-appearing (non-endothelialized) and non-blue (endothelium-covered) areas were counted for the whole aorta, 600-800 points per aorta. The ratio of non-blue to total (blue plus non-blue) points gave an estimate of endothelial regrowth which was expressed on a percentage basis (%ECR). To confirm that the balloon method removed the entire aortic endothelium, at several times during the study rats were injected with Evans Blue 10 minutes after de-endothelialization and then were killed 30 minutes later. In these animals, the entire luminal surface of the aorta was stained blue.

To evaluate intimal thickness, a 1-μm-thick cross-section from each of the four anatomic areas of each rat was quantified with a Nikkon light microscope, a 40× objective, and a projection head. The screen of this head incorporated a coherent multipurpose lattice (Weibel, 1973) with 168 test points spaced 0.9 mm apart. The total number of points falling on profiles of the intima and media were determined for each cross-section by quantifying sequential non-overlapping fields. The ratio of intimal to medial points gave an estimate of the relative volumes of the two tissue compartments (I/M ratio). The ratio of intimal to total counts, expressed as percentage, gave an estimate of the portion of the vessel wall which the intima constituted (%I).

Table 1 Intimal Thickness and Endothelial Regrowth at 2 Weeks Post-Injury, by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>%I (±SEM)</th>
<th>%ECR (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I(Hypox + thyroxin, Corticosterone, DOCA)</td>
<td>13.2 ± 0.12</td>
<td>89 ± 1.25</td>
</tr>
<tr>
<td>II(Hypox, above 3, + GH)</td>
<td>12.6 ± 0.09</td>
<td>89 ± 0.46</td>
</tr>
<tr>
<td>III(Hypox + peanut oil)</td>
<td>11.5 ± 0.10</td>
<td>90 ± 0.56</td>
</tr>
<tr>
<td>IV(Hypox only)</td>
<td>12.3 ± 0.13</td>
<td>92 ± 0.38</td>
</tr>
<tr>
<td>V(Control)</td>
<td>21.7 ± 0.16</td>
<td>79 ± 0.7</td>
</tr>
<tr>
<td>VI(Control + peanut oil)</td>
<td>22.3 ± 0.21</td>
<td>80 ± 0.5</td>
</tr>
</tbody>
</table>
Statistical Analysis

Data consisted of (1) blue (dark) and non-blue (white) counts (percent endothelial cell regrowth, %ECR) and (2) intimal and medial counts (percent intima, %I). Each was analyzed by animal and treatment group, and %I by anatomic area. By analysis of variance techniques, variations due to anatomic location were shown to be small relative to biological variation. Therefore, data from the four cross-sections in each animal were pooled to produce animal means which were then analyzed by group for differences due to treatment, using multiple sample comparison techniques. Duncan’s test was used if the data were normally distributed, as determined by histogram and/or the W-statistic. The Kruskal-Wallis test was used if they were not (Steele and Torrie, 1960; Hollander and Wolfe, 1973; Shapiro and Wilk, 1965).

Results

Body Weight and Aortic Length

All rats had an initial weight of 400 ± 25 g. All lost weight after the de-endothelialization procedure and then stabilized, and there was no statistically significant difference between the hypophysectomized and control groups at the time of death. Aortic lengths and renal weights varied over a fairly wide range, again with no significant difference between pooled hypophysectomized and control animals. However, testicular weights did show a clear difference when the two groups were compared, significant at P < 0.0001. Testicular weights were always 2.5-3.5 g in control animals and 0.8-1.5 g in hypophysectomized animals. Serum cortisol levels obtained at random confirmed that the low-testicular-weight animals were hypophysectomized and the higher testicular weight rats were not.

Effect of Hormone Supplementation and Inert Vehicle Injections on Intimal Thickening and Endothelial Cell Regrowth

Intimal thickness (expressed as %I) was quantified in animals killed 2 weeks after aortic de-endothelialization, and results are given in Table 1. There was no statistically significant difference between any of the four groups of hypophysectomized rats, or between the two control groups. Further, there was no difference between the hypophysectomy groups in endothelial cell regrowth, and there was also no statistical difference between the two control groups; that is, there was no alteration in either intimal proliferation or in endothelial regrowth secondary to the injection of the peanut oil vehicle or to the hormones used.

Endothelial Cell Regrowth

Percent re-coverage of intima by endothelium was compared between hypophysectomized and intact rats, 1 and 2 weeks after balloon de-endothelialization (Fig. 1). At 1 week, endothelial cell coverage was 64 ± 0.89% in hypophysectomized rats and 54 ± 0.57% in control rats (different at P < 0.001). At 2 weeks, coverage was 90 ± 0.18% in hypophysectomized and 79 ± 0.33% in control rats (P < 0.001). In sham-operated rats, the endothelium covered the intima completely. Four weeks after ballooning, both hypophysectomized and control rats showed 98% ECR. Re-coverage of intima by endothelium, then, was greater in hypophysectomized than in control rats at both 1 and 2 weeks, indicating that endothelial cell regrowth was accelerated in hypophysectomized rats.

Intimal Thickening

There were no differences in medial thickness between animals, anatomic areas, or groups. There

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** En face view of aortas 2 weeks post-balloon de-endothelialization. Control (C), top, shows less endothelial regrowth (unfilled arrow) and more areas in which denuded intima remains unrecovered (filled arrow, dark areas). Hypophysectomized rat (H) demonstrates much greater overall endothelial regrowth (unfilled arrow).
were no statistically significant differences in %I between anatomic areas or between individual rats in each group. There was a clear difference in %I, however, between hypophysectomized and control rats at both 1 and 2 weeks. The %I at 1 week was 11 ± 0.22% in hypophysectomized and 14 ± 0.22% in control rats, significant at P < 0.001; and, at 2 weeks, 13 ± 0.038% in hypophysectomized and 22 ± 0.35 in control rats, significant at P < 0.001. For comparison, %I in sham-operated (non-de-endothelialized) rats was 11 ± 0.17%. The intima in these animals consisted of the single layer of endothelial cells, sparse sub-endothelial matrix, and the internal elastic lamina.

Endothelial Cell Regrowth vs. Intimal Thickening

There appears to be an inverse relationship between intimal thickness and percent endothelial regrowth, as seen in Figure 2 (results at 2 weeks). On examination of the data from all animals at 1 and 2 weeks, increased endothelial cell regrowth is associated with less intimal thickening. By linear regression analysis of individual points, this relationship was significant at P < 0.001. Intimal thickening after balloon de-endothelialization, as has been observed previously (Ross and Glomset, 1973), was morphologically due almost completely to smooth muscle cells. To determine if the decreased intimal proliferation was secondary to an increased rate of endothelial cell coverage, the following observations were made. If smooth muscle cell proliferation were totally dependent on the presence or absence of endothelial cells, then areas that remained uncovered by endothelium would be equally thick in hypophysectomized and control animals. Also, areas covered by endothelium would have equivalent intimal thickness in hypophysectomized and control rats. Intimal thickness (%I) was, therefore, quantified in blue (non-re-endothelialized) and non-blue (endothelium-covered) regions of vessels from control and hypophysectomized animals at 1 and 2 weeks. Results are shown in Table 2. Although intimal thickness was slightly greater in each case in control, compared to hypophysectomized, animals, this difference was statistically significant only in re-endothelialized areas two weeks after injury. This suggests that, in addition to accelerating the rate of endothelial cell regrowth, hypophysectomy separately decreases smooth muscle cell proliferation.

Discussion

Four major findings emerge from this study. First, when the balloon catheter model is used (Baumgartner, 1963), hypophysectomy decreases the intimal thickening that occurs after endothelial removal. This is an incomplete suppression, however, as there are areas in which smooth muscle cell proliferation does occur. Second, endothelial cell regrowth after removal is accelerated in hypophysectomized as compared to control rats. This is the first demonstration of alteration of endothelial cell growth in vivo. Unlike the effect on smooth muscle cells, the effect of hypophysectomy on endothelial cells is to increase the rate of re-coverage.

The administration of certain known hormones has no measurable effect on the alterations of intimal thickening and endothelial cell regrowth observed in hypophysectomized animals. It is not likely that the hormones administered were inactive or given in inadequate amounts. The preparations all have been shown to be biologically active in rats, and the dosages and routes of administration have been shown to give adequate replacement levels (Reichlin, 1960; Hess and Riegle, 1972; Denckla, 1973; Denckla, 1974). The failure of these hormones to alter the effects of hypophysectomy was somewhat surprising because of two prior in vitro studies. Gospodarowicz (1974) showed that fibroblast growth factor extracted from brain or pituitary was dependent on corticosterone for stimulation of 3T3
cell proliferation. Kaplan et al. (1979) demonstrated that stimulation of fibroblast growth by platelet-derived growth factor was dependent on the somatomedins, which are produced in response to growth hormone. Thus, the in vivo effect of hypophysectomy is either not related directly to the platelet-derived growth factor or, more broadly, is a different biological effect(s) than that observed in cell culture studies.

Fourth, the results suggest that hypophysectomy has two distinct effects on arterial wall healing after de-endothelialization. Hypophysectomy not only accelerates the rate of regeneration of endothelial cells, it also leads to a clear suppression of smooth muscle cell accumulation in the intima. This was demonstrated by the marked decrease of intimal thickening 2 weeks after endothelial removal in non-re-endothelialized areas in hypophysectomized as compared to intact control rats. It is, then, interesting to consider recent work (Haudenschild and Schwartz, 1979; Schwartz et al., 1979) which indicates that smooth muscle cell proliferation depends strongly on endothelial cell re-coverage. By careful analysis of rat thoracic aortas after balloon de-endothelialization, Haudenschild and Schwartz (1979) demonstrated that, if an area was re-covered within 12 days, no intimal thickening occurred. However, if re-coverage of an area did not occur until 21 days, SMC proliferation occurred. The statistically significant difference in intimal thickness we observed between hypophysectomized and control rats (13 vs. 22% at 2 weeks, $P < 0.01$) is best explained by considering both factors. That is, there is direct suppression of smooth muscle cell proliferation, as seen most clearly in non-endothelialized areas but probably reflected also in the minor differences in intimal thickness between the two groups in the re-endothelialized areas (Table 2).

Second, the increased rate of recovery of intima by endothelium suppresses smooth muscle cell proliferation and leads to a decrease in the area in which intimal thickening occurs and thus to a decrease in total intimal thickness. These two pituitary effects may be related; that is, it remains an open question whether increased rate of endothelial cell regrowth is related directly to the pituitary or is secondary to alterations in the intimal milieu that are caused by the pituitary.

This study, then, is pertinent to recent work concerning growth factors and their modulation (Gospodarowicz, 1974; Antoniades and Scher, 1977; Ross and Vogel, 1978; Shepard, 1980). It demonstrates that the intact pituitary suppresses endothelial cell regrowth and facilitates intimal thickening in the young adult male rat following endothelial removal. The findings also are interesting in reference to the clinical observation that the absence of pituitary function may be associated with decreased atherosclerosis (Merimee, 1978). The exact nature and focus of the pituitary effects remain to be elucidated. That is, it is not clear whether the acceleration of endothelial cell re-growth after hypophysectomy is due to the removal of suppressing factor(s), or merely alteration of the intimal milieu.

**Table 2. Intimal Thickening (% ± SEM) in Areas Not Covered by Endothelium Compared to Areas Re-covered by Endothelium**

<table>
<thead>
<tr>
<th></th>
<th>Re-endothelialized (non-blue)</th>
<th>Non-re-endothelialized (blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Control</td>
<td>11 ± 0.29%</td>
<td>11 ± 0.5%</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td>8 ± 0.18%</td>
<td>10 ± 0.13%</td>
</tr>
<tr>
<td>$P$</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant.

**References**


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