Reduction of collagen biosynthesis in blood vessels and other tissues by reserpine and hypophysectomy

(hypertension/growth hormone)

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ABSTRACT Synthesis of collagen in the vasculature and other tissues of normotensive rats is markedly reduced by reserpine. Hypophysectomy produces similar effects. The effects of reserpine on collagen biosynthesis may be mediated through the hypothalamus.

In a previous report we showed that hypertension causes an increase in collagen biosynthesis and that this increased synthesis can be reversed by the antihypertensive drugs reserpine and chlorthalidone (1). The hypertensive properties of reserpine have been attributed to its depletion of catecholamines in the sympathetic nervous system (2). However, reserpine is also known to have profound effects on endocrine systems (3). This paper represents evidence that reserpine markedly diminishes the synthesis of collagen in the vasculature and in other tissues of normotensive rats and presents additional evidence that the effect may be mediated through the hypothalamus.

MATERIALS AND METHODS Normal and hypophysectomized male rats (8-10-weeks-old) were supplied by Carworth Farms (Wistar strain) and Zivic-Miller, Inc. (Sprague-Dawley strain). The rats were maintained either on a regular laboratory diet and 1% NaCl or on a low iodine diet and 5% sucrose (4). Although the pituitary gland frequently regenerates and restores its function after an incomplete hypophysectomy, both the body and adrenal weights in the ablated rats decreased, indicating the efficacy of the hypophysectomy. Male bilaterally adrenalectomized rats (10 weeks old) were supplied by Carworth Farms (Wistar strain) and maintained on a regular laboratory diet and 1% NaCl. When used, reserpine was administered by daily intraperitoneal injections. Bovine growth hormone (kindly supplied by K. Gibson, Roche Institute of Molecular Biology) was injected subcutaneously into some of the hypophysectomized rats daily for 2 weeks (0.5 mg/kg).

Blood pressures were monitored directly by the tail cuff and photoelectric method without anesthesia. Rats were killed by decapitation and the aorta, mesenteric artery, heart, lung, kidney, liver, and skin were excised and frozen immediately.

Tissue homogenates were prepared in a Polytron St-10 system or a ground glass homogenizer (mesenteric artery) after the addition of 20-30 volumes of 0.25 M sucrose containing 10 mM Tris-HCl (pH 7.4), 100 μM dithiothreitol, and 10 μM EDTA. Prolyl hydroxylase (EC 1.14.11.2) activity was measured in 15,000 × g supernatants by the tritium release assay of Hutton et al. (5) as described by Fuller and Langner (6). The incorporation of [14C]proline into collagenase-digestible protein was determined on pooled samples (100-150 mg of minced tissue) in 1.25 ml of Earle's balanced salt solution buffered with 28 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes) containing 10 μCl of [14C]proline (New England Nuclear Corp.). The incubations were carried out for 5 hr at 37°C under 95% O2 and 5% CO2. After that, the samples were chilled in ice water and the tissues were homogenized in a ground glass homogenizer in 1-3 ml of 0.05 M Tris-HCl (pH 7.4). The homogenates were then dialyzed for 48 hr against four changes of the same buffer. Collagenase-digestible protein was determined by incubating the dialyzed homogenates with purified collagenase (Advanced Biofactures, Lynbrook, N.Y.) for 2 hr at 37°C (7).

Hydroxyproline was measured in aorta homogenates and in urine by the method of Kivirikko et al. (8) after hydrolysis with an equal volume of concentrated HCl. The aortic values for hydroxyproline yield collagen content when multiplied by 6.98 (9). Protein concentration was determined by the method of Lowry et al. (10) with bovine serum albumin as standard.

RESULTS The chronic administration of reserpine at a dose of 0.75 mg/kg was previously shown to reduce blood pressure as well as several markers of collagen biosynthesis in hypertensive rats (1). Fig. 1 shows that there is a dose-related response of reserpine on aortic prolyl hydroxylase (one of the markers of collagen biosynthesis). A dose of 0.75 mg/kg intraperitoneally daily for 2 weeks markedly reduced the blood pressure and resulted in an 80% inhibition of aortic prolyl hydroxylase activity, but did not produce gross behavioral changes in the animals.

The time course for the inhibition of aortic prolyl hydroxylase activity by reserpine (0.75 mg/kg intraperitoneally daily) is shown in Fig. 2. Prolyl hydroxylase inhibition becomes significant between the third and seventh days and the effects of reserpine on blood pressure may even precede the inhibition of prolyl hydroxylase.

Because one of the sites at which reserpine acts is the pituitary, we compared the effects of reserpine on collagen biosynthesis with those of hypophysectomy. Table 1 shows that 2 weeks following hypophysectomy there was a marked decrease in prolyl hydroxylase activity in all tissues examined. Liver and kidney showed similar changes. However, the greatest decreases in enzyme activity were in the aorta, mesenteric artery, and skin. The decreases in enzyme activity following hypophysectomy were greater than those resulting from reserpine treatment. It is of interest to note that treatment of hypophysectomized animals with reserpine resulted in a greater diminution in prolyl hydroxylase activity than either treatment alone, suggesting that reserpine might not be acting solely through its effect on the pituitary.

Adrenalectomized rats were also used to determine the ef-
controls, P < 0.01. The present findings raise the possibility that at least part of the antihypertensive effect of reserpine may be due to its interference with the deposition of collagen and perhaps other components of connective tissue. It has been proposed (12) that the onset and development of hypertension involves, first, humoral or neuronal influences, followed by changes in the elasticity of the larger vessels and an increase in peripheral resistance. Our studies and those of Wolinsky (13, 14) indicate that the vascular changes are most likely due to increased connective tissue deposition, a common response of tissues to insult or injury. Reserpine may represent a drug that influences both the primary humoral events that initially lead to hypertension and the fibrotic vascular sequelae of hypertension.

It is interesting to speculate as to how reserpine diminishes collagen biosynthesis. The biogenic amines norepinephrine and dopamine are capable of causing release of growth hormone from the anterior pituitary (15, 16). Thus, reserpine, which releases and depletes both of these biogenic amines from central sites, may exert its effect on collagen synthesis through these amines, which are involved in the release of growth hormone. The findings with hypophysectomized animals and the replacement studies with growth hormone indicate that the anterior pituitary exerts a profound effect on collagen biosynthesis. The relationship between growth and collagen synthesis

Table 1. Reduction of prolyl hydroxylase activity in rat tissues by reserpine and hypophysectomy

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Prolyl hydroxylase activity (cpm/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>Mesenteric artery</td>
</tr>
<tr>
<td>Control</td>
<td>14,160 ± 648</td>
</tr>
<tr>
<td>Reserpine</td>
<td>7,898 ± 934*</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>2,230 ± 385*</td>
</tr>
<tr>
<td>Hypophysectomy +</td>
<td></td>
</tr>
<tr>
<td>Reserpine</td>
<td></td>
</tr>
</tbody>
</table>

Eight-week-old male Wistar rats were hypophysectomized and 1 week later some of the hypophysectomized rats and controls were treated with reserpine (0.75 mg/kg, intraperitoneal daily) for an additional week. The animals were then killed and the aortas were assayed for prolyl hydroxylase activity as described in Materials and Methods. Values are the mean ±SEM of five rats.

* Statistically different from control, P < 0.01.
† Statistically different from control, P < 0.05.

The effects of the adrenal steroids on prolyl hydroxylase activity. These rats showed no statistically significant decrease in aortic prolyl hydroxylase activity.

Table 2 shows that hypophysectomy not only affects prolyl hydroxylase activity, but also inhibits other markers of collagen synthesis, including incorporation in vitro of [14C]proline into collagenase-digestible protein, the net collagen content of the aorta, and the urinary excretion of hydroxyproline. Table 2 also shows that when growth hormone was administered to the hypophysectomized animals for 2 weeks all the markers of collagen synthesis were partially restored towards normal levels.

DISCUSSION

We have previously shown that hypertension is accompanied by an increase in vascular collagen biosynthesis and that there may be a causal relationship between these two phenomena (1, 11). We have also demonstrated that administration of anti-hypertensive drugs diminishes vascular collagen biosynthesis concomitant with lowering blood pressure. One of the more potent antihypertensive agents, reserpine, was also found to be an unusually potent inhibitor of collagen synthesis. The most common explanation for the antihypertensive action of reserpine has been its depleting effect on neurotransmitter amines, both centrally and peripherally.

![FIG. 1. Dose–response relationship between reserpine and aortic prolyl hydroxylase activity. Eight-week-old male Wistar rats were given various amounts of reserpine, daily by intraperitoneal injection for 2 weeks. The aortas were excised and assayed; each point represents the mean ±SEM for five rats. * Statistically different from controls, P < 0.01.](image)

![FIG. 2. Time course of the effect of reserpine on aortic prolyl hydroxylase activity. Reserpine (0.75 mg/kg) was administered by daily intraperitoneal injection to 6-week-old male Wistar rats. The mean blood pressures are shown within each bar and represent mm of Hg (1 mm of Hg = 133 Pa). Each value of prolyl hydroxylase activity represents the mean of values for five rats. Control rats are represented by the open bars, while the reserpine-treated rats are represented by the hatched bars. * Statistically different from controls, P < 0.01.](image)
is well known and urinary hydroxyproline is used as an index of growth hormone activity in children (17).

Reserpine has also been shown to cause a release of adrenocorticotropic hormone (3). However, the findings with the adrenalectomized rats suggest that the effect of reserpine on collagen biosynthesis is not mediated through action on adrenal steroids.

It is highly unlikely that reserpine itself, or a metabolite of reserpine or some endogenous inhibitor induced by reserpine, could be inhibiting the enzymes necessary for collagen synthesis, and this possibility was experimentally eliminated by the following experiment. In vitro studies with purified enzyme preparations demonstrated that reserpine itself was not an inhibitor of prolyl hydroxylase nor was there an inhibitor of prolyl hydroxylase present in tissues from reserpine-treated animals. It must be concluded, therefore, that the influence of reserpine on collagen biosynthesis is mediated by humoral substances such as biogenic amines and/or growth hormone. It will be of interest to determine whether patients receiving reserpine exhibit any changes in collagen metabolism consistent with these findings.


Table 2. Effect of hypophysectomy and subsequent treatment with growth hormone on some parameters of collagen synthesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[14C]Proline incorporation into collagen (cpm/mg of protein)</th>
<th>Prolyl hydroxylase activity in aorta (cpm/mg of protein)</th>
<th>Collagen content in aorta (mg/g of tissue)</th>
<th>Hydroxyproline in urine (mg in 24 hr/100 g of body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mesenteric artery 6,398 Skin 3,812 Lung 4,608</td>
<td>7,587 ± 950*</td>
<td>63.4 ± 3.3</td>
<td>0.31 ± 0.07</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>3,098 Skin 934 Lung 2,411</td>
<td>917 ± 102*</td>
<td>43.0 ± 2.9*</td>
<td>0.20 ± 0.06</td>
</tr>
<tr>
<td>Hypophysectomy + growth hormone</td>
<td>4,269 Skin 2,847 Lung 3,816</td>
<td>4,346 ± 663†</td>
<td>45.1 ± 2.1</td>
<td>0.29 ± 0.08</td>
</tr>
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Eight-week-old Sprague-Dawley rats were hypophysectomized. At 9 weeks of age growth hormone (0.5 mg/kg subcutaneous) was injected daily for 2 weeks, after which the animals were killed. Measurement of [14C]proline incorporation into collagen represents collagenase-digestible protein, measured as described in Materials and Methods. These values cannot be treated statistically because the determinations were done using minced tissue pooled from 5 rats. The other values are mean ±SEM of five rats.

* Statistically different from control, P < 0.01.
† Statistically different from hypophysectomized rats, P < 0.01.