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# Increased Collagen Synthesis in Blood Vessels of Hypertensive Rats and Its Reversal by Antihypertensive Agents

**(prolyl hydroxylase/hypertension/reserpine)**

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**ABSTRACT** **Collagen synthesis is increased in the aortas, mesenteric arteries, and to a lesser extent, in the hearts of rats either made hypertensive with desoxycorticosterone acetate-salt or that are spontaneously hypertensive. Several markers of collagen biosynthesis were shown to be increased, including prolyl hydroxylase (EC 1.14.11.2; proline, 2-oxoglutarate dioxygenase), prolyl hydroxylase-related antigen, total collagen content, and the incorporation of [$^3$H]proline into total protein and into collagen. The antihypertensive agents chlorothiazide and reserpine, when administered before the onset of hypertension in the rats treated with desoxycorticosterone acetate-salt, prevented or diminished the increase in collagen biosynthesis. When reserpine was given after the onset of hypertension, prolyl hydroxylase activity was decreased concomitant with the decrease in blood pressure. Treatment with reserpine is particularly effective in diminishing arterial prolyl hydroxylase activity.**

The long-term effects of hypertension and atherosclerosis are similar in that both diseases result in a form of vascular wall fibrosis (1–4). Although the anatomic consequences of hypertension involve arterioles primarily, the aorta and other large arteries become dilated with fragmented elastic laminas and increased collagen (5). Since these are age-related changes associated with susceptibility to atherosclerosis, these changes alone may provide an explanation for the well-documented relationship between hypertension and atherosclerosis (5, 6).

Recently, workers in several laboratories have reported increased biosynthesis of collagen in experimental atherosclerosis (3, 7, 8). The availability in this laboratory of methods for measuring a number of markers of collagen biosynthesis has made it possible to determine the effects of hypertension on collagen biosynthesis in blood vessels. We have found that the mesenteric artery and aorta of hypertensive rats, even in the absence of vascular lesions, exhibit a several-fold increase in the formation of collagen chains and in the enzyme prolyl hydroxylase (EC 1.14.11.2; proline, 2-oxoglutarate dioxygenase). These two markers of collagen biosynthesis were decreased towards normal values by antihypertensive agents concomitant with the decrease in blood pressure.

*Materials and Methods.* Desoxycorticosterone acetate was purchased from Nutritional Biochemicals Corp.; reserpine and chlorothiazide were from the Sigma Chemical Co. and Merck and Co., respectively. Bacterial collagenase, free of nonspecific proteases, was obtained from Advance Biofactures, Lynbrook, N. Y.

Desoxycorticosterone acetate-salt hypertension (9,.10) was produced in uninephrectomized, 8-week-old, male Wistar rats by twice-weekly subcutaneous injection of DOCA (5 mg/kg). Rats were maintained on a standard laboratory diet and allowed free access to 1% NaCl drinking water. Normotensive controls were intact, male Wistar rats maintained on a standard laboratory diet and water. Spontaneously hypertensive rats were selected from the available males of uniform age generated by the Hoffmann-La Roche colony described (11, 12). Normotensive controls for these spontaneously hypertensive rats were genetically related Wistar–Kyoto male rats, whose breeding stock was obtained from Dr. Carl T. Hansen, Genetics Unit, Animal Production Section, N.I.H., Bethesda, Md. Blood pressure was monitored at least twice weekly by the tail cuff microphone method, with an instrument made by F. Hoffmann-La Roche & Co., Ltd., Basle, Switzerland.

Each rat was killed by decapitation. The entire aorta, mesenteric artery, and heart were excised, and perivascular adipose tissue was carefully removed. Homogenates of arterial tissue were prepared by compacting the frozen artery into a pellet, adding 30 volumes of 0.25 M sucrose containing 10 mM Tris·HCl (pH 7.4), 100 $\mu$M dithiothreitol, and 10 $\mu$M EDTA, and homogenizing in a Polytron ST-10 (aorta) or a ground glass homogenizer (mesenteric artery). Hearts were homogenized directly in 10 volumes of the above buffer in a Polytron ST-10 homogenizer. Enzyme assays were performed with whole homogenates or a 15,000 $\times$ *g* supernate.

Prolyl hydroxylase activity was measured by the tritium release assay of Hutton *et al.* (13), as described (14). The same substrate was used in all experiments. Total prolyl hydroxylase-related antigen was determined by the enzyme-immunoassay of Stassen *et al.* (15). The incorporation of [$^3$H]proline into protein of aorta and heart was determined *in vitro* with tissue minces incubated in Krebs bicarbonate buffer with 10 $\mu$Ci L-[4-$^3$H]proline. Incorporation of proline into total protein was determined on individual samples (10–100 mg of tissue mince) incubated for 3 hr at 37°. Protein was precipitated by the addition of cold trichloroacetic acid. The pellet obtained by centrifugation was washed three times with the cold 5% acid, and the protein was collected on Millipore filters and counted by liquid scintillation spectrometry. In some experiments, aorta and heart tissue from each group of rats were pooled (100–250 mg of tissue mince) and incubated;

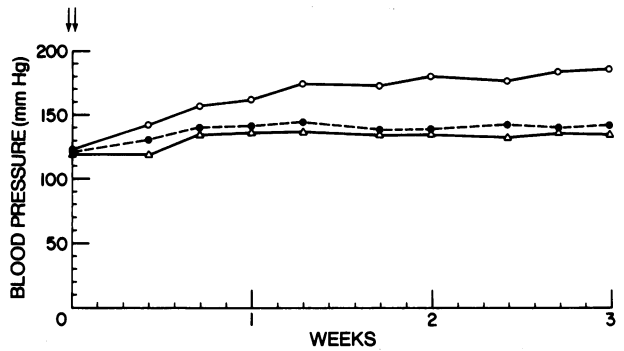


FIG. 1. Uninephrectomized male rats (8 weeks old) were given desoxycorticosterone acetate (5 mg/kg) twice weekly and maintained on saline (○). Concomitant with desoxycorticosterone treatment (see *arrows*), reserpine (△, 0.75 mg/kg) or chlorothiazide (●, 20 mg/kg) was administered daily by intraperitoneal injection. Each point represents the mean of values for 5 rats.

labeled protein was collected as described above. Collagenase-degradable protein was then determined by incubating identical aliquots of protein pellets, precipitated with trichloroacetic acid, and washed with ether, with purified collagenase (16). In this assay, a decrease in the radioactivity remaining in the trichloroacetic acid-insoluble protein, after collagenase treatment, represents the synthesis of collagen *in vitro*.

Hydroxyproline was measured in the whole homogenates by the method of Kivirikko *et al.* (17), after hydrolysis in 6 M HCl. These values yield collagen content when multiplied by 6.98 (18). Protein concentration was determined by the method of Lowry *et al.* (19), with bovine serum albumin as the standard. Tissue sections from each experiment were prepared for histological examination. DNA was determined by the method of Burton (20).

## EXPERIMENTAL

Experiments with desoxycorticosterone acetate-salt hypertensive rats are presented first because severe hypertension is rapidly induced (9, 10). The blood pressures of the treated animals were markedly elevated over those of the controls

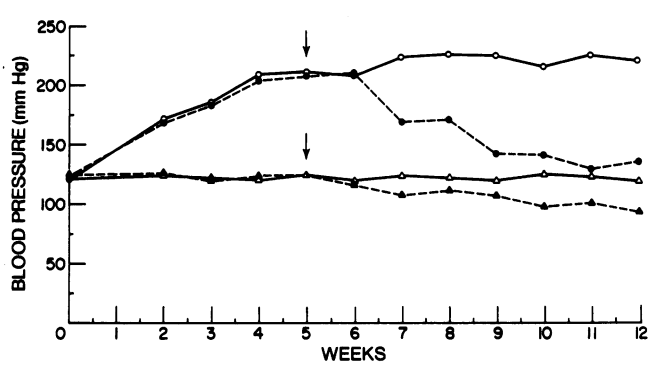


FIG. 2. Uninephrectomized male rats were given desoxycorticosterone acetate (5 mg/kg) twice weekly and maintained on saline. Daily intraperitoneal injection of reserpine (0.75 mg/kg) was initiated 5 weeks after the start of desoxycorticosterone-salt treatment (see *arrows*). Each point represents the mean of values for 5 rats. Rats treated with desoxycorticosterone-salt (○); plus reserpine (●). Normotensive rats (△); plus reserpine (▲).

TABLE 1. *Effect of hypertension caused by desoxycorticosterone acetate-salt on some parameters of collagen biosynthesis*

| | Prolyl hydroxylase activity* | Prolyl hydroxylase-related antigen* | [$^3$H]Proline* |
|---|---|---|---|
| | *Aorta* | | |
| Control | 137 ± 21 | 3,603 ± 484 | 74 ± 12 |
| Hypertensive | 185 ± 34 | 8,311 ± 955† | 168 ± 28† |
| | *Mesenteric artery* | | |
| Control | 144 ± 20 | 2,785 ± 475 | 113 ± 14 |
| Hypertensive | 453 ± 188 | 12,003 ± 2,587† | 475 ± 145† |
| | *Heart* | | |
| Control | 170 ± 7 | — | 70 ± 6 |
| Hypertensive | 199 ± 15 | — | 69 ± 8 |

Control blood pressures were 121 ± 3; hypertensive blood pressures were 227 ± 12†. Rats were made hypertensive by 6 weeks of treatment with desoxycorticosterone-salt.

* Values are Mean ± SEM of 5 rats; expressed as cpm/mg of tissue.

† Statistically different from control, $P < 0.05$.

—, not done.

(Table 1). Concomitant with this, three indicators of collagen synthesis were increased in aorta and mesenteric artery. These were prolyl hydroxylase activity, prolyl hydroxylase-related antigen, and incorporation of [$^3$H]proline into protein. There was little increase in these parameters in the heart.

To see whether the increase in collagen biosynthetic activity was related to the hypertension or to the desoxycorticosterone acetate-salt treatment itself, we administered antihypertensive agents along with the desoxycorticosterone acetate-salt. Two different agents, chlorothiazide and reserpine, were used.

TABLE 2. *Changes in cardiovascular prolyl hydroxylase in rats treated with desoxycorticosterone-salt with and without antihypertensive agents*

| Tissue | Treatment | Prolyl hydroxylase activity |
|---|---|---|
| Aorta | DOCA-salt | 4033 ± 1244 |
| | DOCA-salt + chlorothiazide | 1776 ± 817 |
| | DOCA-salt + reserpine | 96 ± 51* |
| | Control | 1475 ± 341† |
| Mesenteric artery | DOCA-salt | 2318 ± 392 |
| | DOCA-salt + chlorothiazide | 1649 ± 241 |
| | DOCA-salt + reserpine | 870 ± 217* |
| | Control | 1083 ± 38† |
| Heart | DOCA-salt | 3226 ± 217 |
| | DOCA-salt + chlorothiazide | 2624 ± 288 |
| | DOCA-salt + reserpine | 1357 ± 315* |
| | Control | 1695 ± 158† |

The rats were treated as described in *legend* of Fig. 1. Each value represents the mean ± SEM obtained from 5 rats. Values are expressed as cpm/mg of protein. *DOCA*, desoxycorticosterone acetate.

† The control values are the same as those shown in Table 4.

* Statistically different from untreated rats that had been given desoxycorticosterone-salt; $P < 0.05$.

TABLE 3. *Collagen synthesis in vitro in tissues of rats treated with desoxycorticosterone-salt with and without antihypertensive treatment*

| Treatment | [$^3$H]Proline incorporation into protein (cpm/g of tissue) | | [$^3$H]Proline incorporation into collagen (cpm/g of tissue) | | Collagen content (mg/g of tissue) | |
|---|---|---|---|---|---|---|
| | Aorta | Heart | Aorta | Heart | Aorta | Heart |
| DOCA-salt | 26,190 | 7,012 | 6,220 | 385 | 103.3 | 14.7 |
| DOCA-salt + chlorothiazide | 18,400 | 9,664 | 3,100 | 384 | 75.4 | 18.9 |
| DOCA-salt + reserpine | 17,550 | 8,896 | 330 | 288 | 65.9 | 14.7 |

The rats were treated as described in legend of Fig. 1. Values were obtained by assaying pooled tissue samples from 5 rats. [$^3$H]Proline incorporation into collagen represents collagenase-digestible protein, measured as described in *Materials and Methods*. *DOCA*, desoxycorticosterone acetate.

The blood pressures attained during the 3 weeks of treatment were shown in Fig. 1. The prolyl hydroxylase activities in heart and blood vessels of each experimental group are shown in Table 2. Again, the most marked effects of the hypertension occurred in aorta and mesenteric artery, although an appreciable effect was observed this time in the heart as well. The prolyl hydroxylase levels were lowest in all tissues of those animals treated with antihypertensive agents. It is of interest that reserpine-treated animals yielded values that were lower, and in the aortas, much lower than even those of normotensive controls. The activities reported in Table 2 are expressed in terms of mg of protein. The same relationships were observed when they were expressed in terms of units of DNA.

Three other indicators of collagen biosynthesis were measured in the aortas and hearts of these animals. As shown in Table 3, [$^3$H]proline uptake into total protein, [$^3$H]proline uptake into collagen, and even the total collagen of the aortas were higher in the animals treated with desoxycorticosterone acetate-salt than in the animals receiving desoxycorticosterone acetate-salt plus chlorothiazide or reserpine. Again the lowest values were obtained with reserpine. No significant changes were observed in the heart.

If antihypertensive agents can prevent the increase in collagen biosynthesis in blood vessels, can they also reverse the already increased collagen biosynthesis in hypertensive animals? The experiment summarized in Fig. 2 and Table 4 shows that concomitant with the reduction in blood pressure, reserpine brought about a reduction in prolyl hydroxylase. As before, the effects were most marked in the aorta, and the values obtained in the aorta with reserpine-treated animals were significantly lower than in the normotensive controls. It may be noted that there was a considerable decrease in prolyl hydroxylase activity over the 9-week period. This was to be expected since collagen biosynthesis in most rat tissues diminishes with age (15, 21). The present findings indicate that collagen biosynthesis also decreases with age in the cardiovascular system.

The effects of hypertension on vascular collagen biosynthesis were also investigated in spontaneously hypertensive rats. The onset of hypertension in this case is slower and the degree of hypertension is less than in the rats treated with desoxycorticosterone acetate-salt. These studies were also started in young rats at a time when collagen biosynthesis was diminishing. Nevertheless (Table 5), by 8 months of age (240 days) the spontaneously hypertensive animals showed higher levels of prolyl hydroxylase activity in the aorta, mesenteric artery, and the heart than was observed in the normotensive controls.

## DISCUSSION

It appears that hypertension, in itself, leads to an increase in collagen biosynthesis in the vasculature. No doubt this is accompanied by an increase in the other matrix components, elastin and mucopolysaccharide. This response to hypertension is similar to the response of blood vessels to other agents (physical and chemical) that also bring about atherosclerosis

TABLE 4. *Changes in cardiovascular prolyl hydroxylase activity (cpm/mg of protein) on treatment with desoxycorticosterone-salt, with and without reserpine*

| | Desoxycorticosterone-salt | | Control | |
|---|---|---|---|---|
| | − Reserpine | + Reserpine | − Reserpine | + Reserpine |
| *3 Weeks of treatment* | | | | |
| Aorta | 6,188 ± 1,158* | — | 1,475 ± 341 | — |
| Mesenteric artery | 3,122 ± 760* | | 1,083 ± 38 | — |
| Heart | 3,766 ± 209* | — | 1,695 ± 158 | — |
| *12 Weeks of treatment* | | | | |
| Aorta | 4,348 ± 80* | 731 ± 55† | 1,114 ± 102 | 849 ± 190 |
| Heart | 1,038 ± 101* | 684 ± 57† | 753 ± 107 | 706 ± 87 |

The rats were treated as described in *legend* of Fig. 2. Each value is the mean ± standard error of at least 5 rats per group.—, not done.
* Significantly different from normotensive control, $P < 0.05$.
† Significantly different from untreated hypertensive group, $P < 0.05$.

TABLE 5. *Blood pressure and cardiovascular prolyl hydroxylase activity in spontaneously hypertensive rats and controls*

| | Rats | Blood pressure (mm Hg) 50 Days | 120 Days | 240 Days |
|---|---|---|---|---|
| | SHR | 148 ± 2.0* | 203 ± 4.9* | 204 ± 5.8* |
| | Controls | 118 ± 1.5 | 121 ± 3.6 | 122 ± 1.3 |

| Tissue | Rats | Prolyl hydroxylase activity (cpm/mg of protein) 50 Days | 120 Days | 240 Days |
|---|---|---|---|---|
| Aorta | SHR | 5421 ± 557* | 2499 ± 208* | 2118 ± 424* |
| | Control | 3000 ± 473 | 1764 ± 235 | 978 ± 102 |
| Mesenteric artery | SHR | 7654 ± 319 | 3729 ± 631 | 1645 ± 186* |
| | Control | 5578 ± 805 | 2426 ± 520 | 1051 ± 181 |
| Heart | SHR | 1835 ± 111 | 1769 ± 285 | 1491 ± 88* |
| | Control | 1796 ± 118 | 1222 ± 80 | 1197 ± 39 |

Each value is the mean ± standard error obtained on 4 or 5 rats. *SHR*, spontaneously hypertensive rats.
* Statistically different from control, $P < 0.05$.

(see introduction). In the present studies the spontaneously hypertensive rats showed no signs of vascular lesions. Rats treated with desoxycorticosterone acetate-salt for 3 weeks showed mild myocardial fibrosis (2 out of 10 rats). After 6 weeks of treatment with desoxycorticosterone acetate-salt, the rats showed periarteritis nodosa in the mesenteric artery (9 out of 12 rats), thickening of the aorta (11 out of 12 rats), and myocardial fibrosis (7 out of 12 rats). The increase in vascular collagen biosynthesis that is brought on by physical or chemical insults to the vascular system may be compared to the increases in collagen formation brought about by insult or injury to most tissues: skin, liver, lung, etc. (22). The arterial system may normally be undergoing continual damage and repair, requiring an active and well-regulated system for synthesis and degradation of connective tissue. Insults to the vasculature, if intense or prolonged, appear to lead to excessive connective tissue formation. Hypertension appears to be one type of insult that enhances vascular connective tissue formation. It is of interest that the largest increase in collagen biosynthesis was observed in the aorta, which in most species is particularly susceptible to atheroma formation. Values were also enhanced in the mesenteric artery. The low and variable increments in the heart are to be expected, since most of this tissue is muscle; the vasculature makes up only a small portion of the heart. Crossley *et al.* (23) have already shown that the increased collagen biosynthesis brought about by a variety of atherogenic agents, other than hypertension, is limited to the vasculature.

The increased collagen biosynthesis seen in the vasculature of hypertensive rats may explain the thickening and changes in elasticity of blood vessels that are observed in hypertension (2, 24). Increased collagen biosynthesis may also be an early indicator of the vascular lesions brought on by the hypertension. The latter is generalized, the arteriosclerosis localized to areas where the insults (pressure, turbulence) are greatest. Thickening of blood vessel walls and arteriosclerosis may be, in part, sequelae of the hypertension on the repair process of the blood vessels. The effects of chlorothiazide and reserpine on vascular collagen biosynthesis are no doubt related to their lowering of blood pressure.

The beneficial effects of antihypertensive drug therapy on the course of hypertension and the subsequent development of arteriosclerosis has been well documented (25–28). Since the formation of collagen is an important step in the vascular pathology of arteriosclerosis and hypertension, the present findings demonstrate one possible biochemical explanation for the efficacy of antihypertensive drugs in the treatment of cardiovascular disease.


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