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Reduction of blood pressure and vascular collagen in hypertensive rats by β-aminopropionitrile

(hypertension/blood vessels)

KAZUHIKO IWATSUKI, GEORGE J. CARDINALE, SYDNEY SPECTOR, AND SIDNEY UDENFRIEND

Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Contributed by Sidney Udenfriend, October 21, 1976

ABSTRACT **β-Aminopropionitrile, a specific inhibitor of lysyl oxidase prevented the rise in blood pressure induced by deoxycorticosterone-salt in rats. In addition, after the onset of hypertension, administration of β-aminopropionitrile lowered the blood pressure. Concomitant with the lowering of blood pressure, there was a reduction in the more highly crosslinked form of vascular collagen. These findings would indicate that increases in vascular connective tissue are not only sequelae of hypertension, but may also contribute to the maintenance of elevated blood pressure.**

We have previously reported that vascular collagen biosynthesis and deposition is increased in hypertensive rats and that these effects are reversed when blood pressure is lowered by antihypertensive drugs (1, 2). Those findings suggested that the deposition of vascular connective tissue is one of the sequelae of hypertension. The availability of agents that selectively inhibit collagen formation and deposition make it possible to determine whether the increased amounts of collagen also contribute to the maintained hypertension. One of these agents, β-aminopropionitrile, is a fairly specific inhibitor of lysyl oxidase, the enzyme which catalyzes the oxidative deamination of the ε-amino groups of lysine and hydroxylysine in collagen and elastin (3–5). In the absence of lysyl oxidase activity, the formation of crosslinked collagen is blocked. This can be demonstrated by a diminution of insoluble collagen (6).

In the present study we have shown that administration of β-aminopropionitrile to rats receiving deoxycorticosterone-salt markedly diminishes the hypertension produced by the steroid. When β-aminopropionitrile was given after the onset of severe hypertension, it lowered the blood pressure. Concomitant with the changes in blood pressure, there were parallel changes in insoluble vascular collagen.

MATERIALS AND METHODS

Deoxycorticosterone acetate was purchased from ICN Pharmaceuticals, Inc. β-Aminopropionitrile fumarate was obtained from the Aldrich Chemical Co. and was dissolved in 0.9% saline. The dose of β-aminopropionitrile used is expressed in terms of the free base.

Twelve-week-old male Wistar rats, weighing 300–350 g, were subjected to uninephrectomy. The rats were then divided into three groups. One group received subcutaneous injections of deoxycorticosterone (5 mg per rat) twice weekly (7, 8); the second group received the same amount of deoxycorticosterone and in addition either 50 mg/kg or 100 mg/kg of β-aminopropionitrile twice daily, intraperitoneally; the third group received β-aminopropionitrile alone twice daily. Normotensive, intact, male Wistar rats were used as controls. The rats were maintained on a standard laboratory diet and were given 1% saline in tap water *ad libitum*. Blood pressure was measured by the tail cuff and photoelectric method without anesthesia (9).

Rats were killed by decapitation. The entire aorta and the mesenteric artery were excised from each animal and perivascular adipose tissue was carefully removed from them. Each vascular tissue was homogenized in 30 volumes of 0.5 M acetic acid in a ground-glass homogenizer, and the soluble collagen was extracted for 24 hr at 4° (10). The extracts were centrifuged at 11,500 × *g* for 30 min and the supernatants were dialyzed against 10 mM acetic acid. The precipitates, containing the insoluble collagen, were then lyophilized. After this, the supernatants and precipitates were hydrolyzed in 6 M HCl for 20 hr at 105°. Hydroxyproline was measured by the method of Kivirikko *et al.* (11). These values yield collagen content when multiplied by 6.98 (12).

Tissue sections from each experiment were prepared for histological examination.

RESULTS

Changes in blood pressure and body weight

As shown in Fig. 1, the blood pressures of the rats treated with deoxycorticosterone-salt were markedly increased over those of the controls. Administration of β-aminopropionitrile to rats treated with deoxycorticosterone-salt largely prevented the increase in blood pressure for 5 weeks. After this time, the blood pressure gradually increased to about 170 mm of Hg by the fifteenth week of treatment. At this point, however, the blood pressure was still much lower than in the rats treated with deoxycorticosterone-salt alone ($P < 0.01$). When β-aminopropionitrile was given after deoxycorticosterone-salt hypertension was established, the blood pressure was lowered. It should be noted that the fall in blood pressure was not acute but gradual. After 10 weeks, some of the rats that were receiving deoxycorticosterone-salt and β-aminopropionitrile had the β-aminopropionitrile removed from their regimen. Concomitant with the removal of β-aminopropionitrile, the blood pressure increased to 210 mm of Hg over a 1-week period, the same pressure as in those animals that never received β-aminopropionitrile. β-Aminopropionitrile by itself had no effect on blood pressure in normotensive animals.

Fig. 2 shows the blood pressure of rats given 50 mg or 100 mg/kg of β-aminopropionitrile twice daily along with deoxycorticosterone-salt for a 6-week period. It would appear that the lower dose is near the threshold dose for the antihypertensive effect of β-aminopropionitrile. The changes in body weight caused by the various agents used were slight.

Changes in collagen

As shown in Table 1, total and insoluble collagen were significantly increased over controls in the aortas of rats treated with deoxycorticosterone-salt after β-aminopropionitrile was removed from their regimen for 5 weeks. Animals that had been treated with deoxycorticosterone-salt and that were treated with

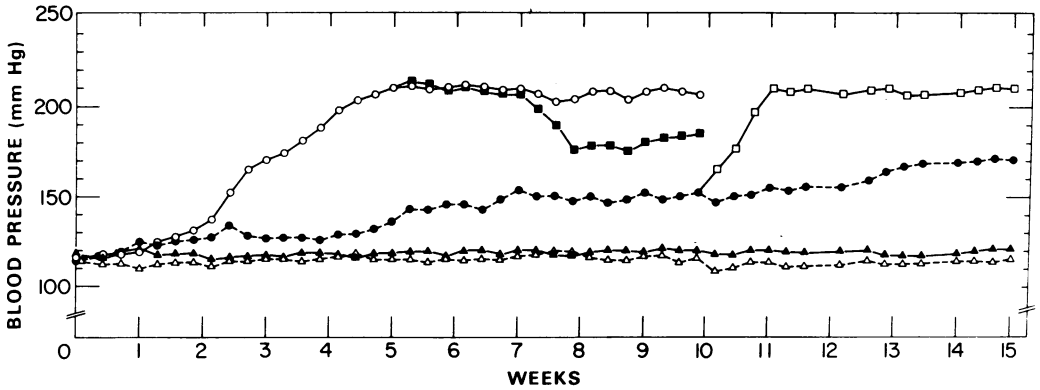

FIG. 1. Uninephrectomized male rats (12 weeks old) were given deoxycorticosterone acetate (5 mg/kg) twice weekly and maintained on saline (○). After 5 weeks of deoxycorticosterone-salt treatment, β-aminopropionitrile (100 mg/kg intraperitoneally) was administered twice daily (■). Another group of rats was given the same dose of β-aminopropionitrile concomitant with the start of deoxycorticosterone-salt treatment (●). After 10 weeks of the combined deoxycorticosterone-salt-β-aminopropionitrile treatment, the β-aminopropionitrile injections were stopped (□). Intact, normotensive rats (▲) and uninephrectomized normotensive rats given the standard dose of β-aminopropionitrile (△) served as controls. At 15 weeks the remaining rats were killed and the collagen content of their tissues was measured (Table 1). Each point represents the mean of values for at least three rats.

β-aminopropionitrile for the entire 15-week period did not show this increase over controls, and, in fact, their collagen content was significantly lower than in the animals in which β-aminopripionitrile treatment had been discontinued. Administration of β-aminopropionitrile to normotensive animals yielded aortas containing the smallest amounts of collagen. Similar results were obtained with mesenteric arteries. In that tissue also, β-aminopropionitrile treatment significantly decreased collagen content.

DISCUSSION

The first step in the crosslinking of collagen and of elastin is the oxidative deamination of lysine and hydroxylysine by the enzyme lysyl oxidase (13, 14). β-Aminopropionitrile inhibits the activity of this enzyme and thereby should decrease the deposition of collagen and elastin fibers.

In previous studies we have shown that hypertension results in an increase in the synthesis and deposition of vascular collagen (1, 2). We have now used β-aminopropionitrile to further investigate the relationship between the onset of hypertension and collagen biosynthesis. As shown in Fig. 1, β-aminopropionitrile can prevent or reverse the hypertension produced by deoxycorticosterone-salt. Concomitant with the decrease in blood pressure, there was a decrease in vascular collagen content (Table 1). Recently, Ooshima and Midorikawa (personal communication) have also shown that β-aminopropionitrile lowers blood pressure concomitant with a decrease in vascular collagen deposition.

As was previously reported (1, 2), periarteritis nodosa in the mesenteric artery and thickening of the aorta were observed in the hypertensive rats after 10 or 15 weeks of treatment. In contrast, blood vessels of rats that had been treated with deoxycorticosterone-salt and with β-aminopropionitrile did not show these changes and, in fact, were similar to those of control rats.

It should be noted that the hypotensive effect of β-aminopropionitrile was not acute. The long onset would be in accord with an effect on collagen synthesis rather than an acute pharmacological effect. One possible pharmacological effect which suggested itself was that β-aminopropionitrile may be lowering blood pressure by inhibiting monoamine oxidase. It is well known that inhibitors of monoamine oxidase are antihypertensive agents (15). Since both lysyl oxidase and mono-

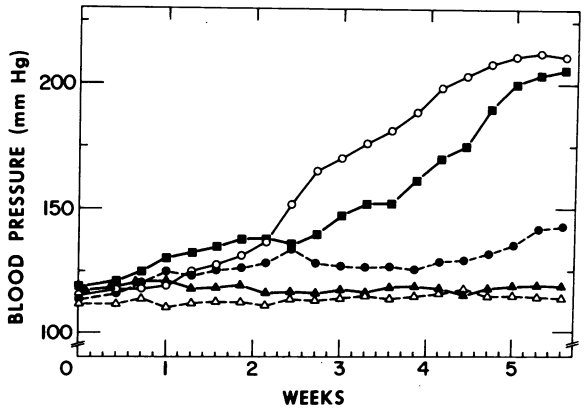

FIG. 2. Uninephrectomized male rats (12 weeks old) were given deoxycorticosterone acetate (5 mg/kg) twice weekly and maintained on saline (○). Concomitant with the deoxycorticosterone-salt treatment, β-aminopropionitrile (50 mg/kg, ■, and 100 mg/kg, ●) was administered twice daily by intraperitoneal injection. Intact, normotensive rats (▲) and uninephrectomized normotensive rats given the high dose of β-aminopropionitrile (△) served as controls. Each point represents the mean of values for at least 10 rats.

Table 1. Collagen content of aorta in rats treated with deoxycorticosterone salt in the presence or absence of β-aminopropionitrile

Treatment	Collagen content (mg/g tissue)	
	Insoluble	Total
Normotensive (5)	115.9 ± 1.5	152.9 ± 3.6
Normotensive + β-amino propionitrile (4)	85.6 ± 1.8*	127.7 ± 1.8*
Deoxycorticosterone salt (3)	131.9 ± 0.07*	172.4 ± 1.8*
Deoxycorticosterone salt + β-aminopropionitrile (4)	94.2 ± 3.5†	136.1 ± 3.5†

The rats were treated for 15 weeks as described in the legend of Fig. 1. The numbers in parentheses represent the number of animals. Each value is the mean ± standard error.

* Significantly different from normotensive control, $P < 0.01$.

† Significantly different from animals treated with deoxycorticosterone salt, $P < 0.01$.

amine oxidase are oxidative deaminases, this possibility had to be examined. One of the most sensitive tests for inhibitors of monoamine oxidase *in vivo* is their ability to increase levels of brain catecholamines (16). When β-aminopropionitrile was compared to iproniazid, a well-known monoamine oxidase inhibitor (17), it was found that treatment for 2 days with β-aminopropionitrile (100 mg/kg twice daily) had no effect on brain catecholamine levels. By contrast, iproniazid at a dose of 75 mg/kg for 2 days doubled brain catecholamine levels.

There have been a number of approaches to the treatment of hypertension. These studies suggest a completely new approach in both the understanding and treatment of hypertension; namely, agents that interfere with deposition of vascular connective tissue. Obviously, such agents can produce toxic manifestations (6, 18, 19). However, it should be remembered that the lathyrism produced by β-aminopropionitrile is a disease limited to young, rapidly growing animals, while the incidence of hypertension in man is mostly in the older population.

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