Digitalis Attenuates Arterial Hypertrophy in Experimental Hypertension (43104)

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Abstract. **Several investigators have reported that digitalis administration reduces cardiac hypertrophy in rats with experimental hypertension. To determine whether digitalis similarly affects growth of arteries, we studied young (5- to 14-week-old), male, one-kidney, one-clip hypertensive rats (1K1C; $n = 14$) and one-kidney normotensive control rats (1K; $n = 26$). Half of the rats received digoxin (150 mg/kg body wt/day) in chow starting 1–2 weeks before clipping (1K1C-D; 1K-D); the other half were pair-fed (1K1C-C; 1K-C). Serum digoxin levels averaging 488 ng/ml were documented in rats receiving digoxin. After 3–5 weeks of hypertension (conscious tail blood pressures), and at a similar time period in normotensive control rats, we measured direct femoral arterial pressure and weighed standardized segments of the thoracic aorta. At sacrifice body weights of the four groups did not differ. In the one-kidney control rats, mean ± SE femoral arterial pressure (1K-D, 108 ± 3; 1K-C, 111 ± 4, mm Hg), thoracic aortic dry weight (1K-D, 36.6 ± 0.6; 1K-C, 36.2 ± 1.1. mg/kg body wt), and aortic water content (1K-D, 62.7 ± 0.4; 1K-C, 62.4 ± 0.4, % wet weight) did not differ between rats receiving or not receiving digoxin, respectively. As compared with pooled normotensive control rats, femoral arterial pressure (1K1C-D, 165 ± 8; 1K1C-C, 153 ± 5), aortic water content (1K1C-D, 64.8 ± 0.4; 1K1C-C, 64.9 ± 0.5), and aortic weight (1K1C-D, 44.8 ± 2.1; 1K1C-C, 50.1 ± 1.6) were increased ($P < 0.001$) in the one-kidney, one-clip rats, on or off digoxin. Comparison of hypertensive rats receiving to those not receiving digoxin revealed no differences in arterial pressure or aortic water content, but aortic growth was significantly attenuated (−41%, $P = 0.02$) in the hypertensive rats receiving digoxin. These results provide evidence that digoxin reduces hypertensive arterial growth by a mechanism that does not affect normal growth.** [P.S.E.B.M. 1990, Vol 195]

Several investigators have observed that digitalis administration reduces cardiac hypertrophy in experimental hypertension (1–4). However, the effect of digitalis on growth of arteries in hypertension has not been studied. Increase in wall thickness in large arteries in hypertension decreases compliance and elevates cardiac work (5). Increase in wall thickness at the arteriolar level may be importantly involved in the elevated resistance underlying hypertension (6). Therefore, the primary purpose of this study was to test the hypothesis that digitalis administration attenuates the growth of arteries accompanying experimental hypertension in rats.

Materials and Methods

Methods were modifications of those we have previously described (7–9). Briefly, male Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA), body weight 150–190 g, age 5–6 weeks, were divided into pairs. One member of each pair received 150 mg of digoxin/kg body wt/day (Crystalline digoxin; Sigma Chemical Co., St. Louis, MO) mixed with his powdered chow; the other member was pair-fed with powdered chow not containing digoxin to maintain his body weight close to that of the paired rat. Chow (PROLAB R-M-H 1000; Agway, Syracuse, NY) contained 0.4% sodium and 0.7% potassium and all rats drank tap water *ad libitum*. After 9–13 days of this diet, all rats were unilaterally nephrectomized by laporotomy. In half the pairs, chosen at random, the contralateral (left) renal artery was also partially constricted with a 0.44-mm ID silver clip to produce one-kidney, one-clip (1K1C) hypertension. The remaining pairs were sham-clipped as one-kidney (1K) normotensive controls. Thereafter, blood pressures (unanesthetized) were measured one to three times per week by tail cuff (Natsume Rat Tail Manometer System, model KN-009, Natsume, Tokyo, Japan). After 3–5 weeks of documented hypertension (systolic blood pressure > 140

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mm Hg) and at age 11–14 weeks in the 1K1C rats, and at a similar time period in the 1K-normotensive control rats, the paired rats were lightly anesthetized with ether and the mean femoral pressure was directly measured. Then arterial blood was obtained from the abdominal aorta for measurement of hematocrit and plasma sodium, potassium, and creatinine. Blood was carefully handled to avoid hemolysis. A standardized segment of the thoracic aorta (left subclavian origin to the diaphragm) was rapidly excised, cleaned of blood and fat, and the wet weight recorded. The thoracic aorta was reweighed after 24 hr at 100°C, at which time we documented a stable dry weight. Difference between wet and dry weight was considered water content and was expressed as percentage of wet weight. Each rat was necropsied, and clip placement, condition of heart, kidney, lungs, as well as general health were assessed.

Terminal digoxin concentrations were measured in plasma from nine rats receiving digoxin and nine control rats (affinity column-mediated immunoassay DGN pack; DuPont, Wilmington, DE). Plasma Na^+, K^+, and creatinine concentrations were measured by flame emission and Sigma kit 555, respectively.

Data were analyzed by one-way analysis of variance and subsequent comparison of individual means was by Newman-Keuls (10). The null hypothesis was rejected at $P < 0.05$.

Results

All rats reported remained in good general health and either gaining or of stable weight. Careful necropsy of 1K1C rats verified the presence of the renal artery clip and good general health. Necropsy of 1K rats revealed no pathology other than compensatory renal hypertrophy.

Terminal plasma digoxin levels in nine rats receiving digoxin (mean ± SE) were 488 ± 68 ng/ml, higher than those we have previously reported (7) for rats receiving 120 mg of digoxin/kg/day. Control levels were 3 ± 1 ng/ml. Terminal hematocrit and plasma Na^+ and creatinine (Table I) did not differ among the four groups. In contrast (Table I), plasma K^+ concentrations were 17–18% lower ($P < 0.001$) in rats receiving digoxin, whether hypertensive or not.

Table I also presents data on blood pressures and organ weights and water contents. (It should be noted that in this table values within a row sharing the same letter are *not* significantly different; values not sharing letters differ ($P < 0.05$) by analysis of variance.) In contrast to our previous study (7), in which 125 mg/kg of digoxin increased blood pressure by 10 mm Hg, we observed no increase in blood pressure with digoxin administration in control normotensive rats in the present study (Fig. 1). This difference may be related to the higher levels of plasma digoxin achieved or to the unilateral nephrectomy in the present study. Development, duration, and magnitude of hypertension also did not differ in 1K1C rats receiving or not receiving digoxin and this is also illustrated in Figure 1. Conscious tail blood pressures, averaged over the 3- to 5-week duration of hypertension, were elevated by 36–37% in 1K1C rats compared with values in normotensive control rats (Table I). Terminal femoral arterial mean blood pressure measured directly under light ether anesthesia confirmed the results of the indirect tail measurements. Terminal body weights did not significantly differ among the four groups. Water content of the thoracic aorta in hypertensive rats was increased by 3.7%, but did not differ in rats receiving or not receiving digoxin. In hypertensive rats not receiving digoxin, dry weight of standardized segments of thoracic aorta, and aortic dry weight normalized to body weight, were increased by 41% and 39%, respectively ($P < 0.001$) compared with 1K control rats. In contrast, in hypertensive rats receiving digoxin, dry weight and normalized dry weight of thoracic aorta were increased by only 22–23% ($P < 0.01$) compared with 1K rats receiving digoxin; this attenuation (−41%, normalized weight) of arterial growth in hypertensive rats by digoxin was statistically significant ($P = 0.02$).

Discussion

Although several investigators have observed that digitalis decreases cardiac hypertrophy in experimental hypertension (1–4), this is the first study to provide evidence that cardiac glycosides also attenuate the growth of arteries, specifically the aorta. Abnormalities in the function of resistance arteries are clearly the basis of the hypertensive process. However, abnormalities in the compliance function of the aorta increase cardiac afterload and work (5), and thus also have an important role in the disease mechanisms of hypertension. Others have found a relationship in hypertension between increases in wall cross-sectional area of conduit arteries, reflecting growth, and reduction in distensibility (11).

Measurements of arterial dry weight, such as we made, have the advantages of simplicity and accuracy. Changes in aortic dry weight, as observed in the present study, clearly reflect changes in bulk tissue composing the aortic wall. However, such measurements do not define the specific nature of the increase in bulk, whether hypertrophy or hyperplasia, and whether smooth muscle or connective tissue. Thus, morphologic or biochemical studies will be necessary to further define the specific growth variables attenuated by digoxin. It does seem likely, however, that in the hypertensive aorta true muscular hypertrophy is involved (12).

Smooth muscle growth and wall thickening occurs in resistance as well as in conduit arteries of rats with Goldblatt hypertension (9). If digoxin also attenuated this growth of arteriolar walls in our hypertensive rats, the effect would be a reduction in arteriolar wall to lumen ratio (6). Tobian and Binion (13) calculated that

Table I. Blood Pressures, Weights, Water Content, Hematocrit, Plasma Na^+, K^+, Creatinine[a]

	1K-C	1K-D	1K1C-C	1K1C-D
n	13	13	7	7
Weeks of hypertension	—	—	3.21 ± 0.29a	3.79 ± 0.38a
BP, tail (mm Hg)	121.3 ± 1.1a	121.6 ± 1.7a	166.1 ± 4.1b	164.9 ± 2.8b
BP, femoral (mm Hg)	111.1 ± 4.4a	108.5 ± 3.4a	152.6 ± 5.2b	164.7 ± 7.8b
BW (g)	413.2 ± 7.7a	421.2 ± 8.9a	420.7 ± 12.1a	427.0 ± 19.7a
TA (mg)	14.95 ± 0.52a	15.41 ± 0.34a	21.07 ± 0.87	18.98 ± 0.66
TA/BW (mg/kg)	36.17 ± 1.09a	36.65 ± 0.60a	50.12 ± 1.61	44.85 ± 2.06
TA H_2O (%)	62.36 ± 0.37a	62.72 ± 0.43a	64.90 ± 0.54b	64.80 ± 0.37b
Hct (vol %)	41.6 ± 0.9a	41.9 ± 0.6a	42.5 ± 0.5a	42.8 ± 1.5a
Creatinine (mg/dl)	0.72 ± 0.05a	0.66 ± 0.05a	0.57 ± 0.07a	0.62 ± 0.05a
Na^+ (mEq/liter)	142.9 ± 1.2a	144.6 ± 1.7a	142.2 ± 1.5a	146.8 ± 3.2a
K^+ (mEq/liter)	3.41 ± 0.08a	2.82 ± 0.08b	3.46 ± 0.11a	2.85 ± 0.14b

[a] BP, blood pressure; BW, terminal body weight; TA, thoracic aortic dry weight; TA H_2O, thoracic aortic water content as % of wet weight; Hct, hematocrit. Values within a row sharing the same letter are *not* significantly different ($P > 0.05$); $n = 12$, for creatinine, Na^+, and K^+ values in 1K-C and 1K-D.

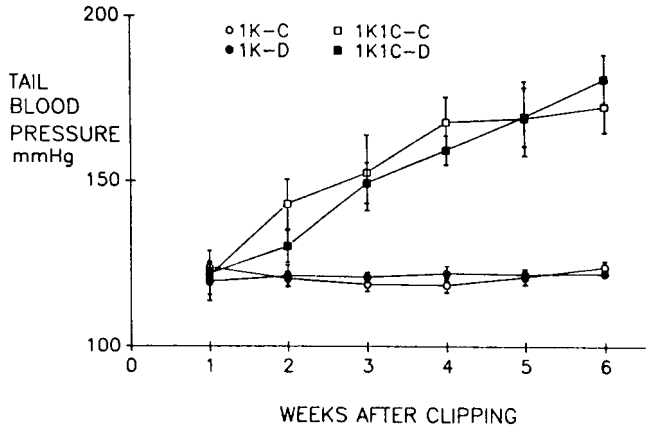

Figure 1. Weekly tail blood pressures (mean ± SE, mm Hg) following clipping or sham clipping (at Week 0). One-kidney normotensive controls, no digoxin (1K-C), ○; one-kidney normotensive controls, receiving digoxin (1K-D), ●; one-kidney one-clip hypertensives, no digoxin (1K1C-C), □; one-kidney one-clip hypertensives, receiving digoxin (1K1C-D), ■.

a generalized 13% change in bulk of the walls of arterioles would change overall flow resistance by as much as 54%. Thus, a generalized decrease in arteriolar wall bulk similar to that occurring in conduit arteries with digoxin administration in our rats should have had an antihypertensive effect; other variables held constant. In this regard, others have observed a mild (12–24%) hypotensive effect of cardiac glycosides in rats with adrenal-compression hypertension (3) and in spontaneously hypertensive rats (14). However, in neither the present study nor in two other previous studies of the effects of digitalis in hypertension (1, 2) was such an antihypertensive effect noted. Clearly, morphologic studies of the effects of digitalis on arteriolar growth in hypertension, as well as studies of functional effects on arteriolar resistance, are needed.

In the present study digoxin administration did not reduce aortic weight in the growing normotensive control rats. Thus, its growth-attenuating effects appeared only in the presence of an abnormal growth stimulus or stimuli. Other investigators reached a similar conclusion for digitalis attenuation of myocardial hypertrophy in hypertension (1). However, neither these previous studies nor this study identify the mechanism(s) by which cardiac glycosides attenuate cardiovascular hypertrophy in hypertension (1–4). The mechanism seems unrelated to effects on pressure components of cardiovascular work. However, effects on flow components are a possibility. Additionally, cardiac glycosides have a general inhibitory effect on the growth of cultured cells (15). Thus, future studies of the effects of digitalis on the growth of cultured cardiovascular cells may reveal mechanisms.

As an additional possibility, digitalis may attenuate growth by reducing the levels or effects of endogenous growth factors. Ayachi and Hall (3) suggested that digitalis may compete with a putative hypertensinogenic steroid for mineralocorticoid receptors in adrenal compression hypertension in rats. Schreiber *et al.* (4) presented evidence for the presence of a cardiotropic factor with digoxin-like immunoreactivity in serum of rats with coarctation hypertension. They observed that serum from coarcted rats almost doubled the rate of division of cardiac myocytes in tissue culture (as compared to the effects of control normotensive serum). They suggested that this digoxin-like factor mainly influences myocardial proteosynthesis and that its inotropic effect is weaker. Importantly, they proposed that administration of exogenous cardiac glycosides reduces secretion of this endogenous factor, thereby attenuating myocardial hypertrophy. This hypothesis, which may also apply to arterial hypertrophy, has not yet been adequately tested, but results of the present investigation provide some support.

Finally, in the present study plasma K^+ concentrations were 17–18% lower ($P < 0.001$) in the rats receiv-

ing digoxin, whether hypertensive or not. We had observed trends toward hypokalemia in our previous study of the effects of digoxin administration to unoperated normal rats (6). To our knowledge, such a hypokalemic effect of digitalis administration has not been previously reported and we have no explanation for this interesting new observation. Although the lower K^+ concentrations had no effect on the variables we measured in control normotensive rats, it is possible that altered K^+ levels in plasma reflected abnormal tissue levels that were somehow involved in the attenuated arterial wall growth we observed in the hypertensives (16).

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