The Vascular Na\(^+\)-K\(^+\) Pump in Experimental Hypertension

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SUMMARY We assessed the role of putative circulating ouabainlike factor(s) on in vivo arteriolar function in rats with very early (<7 days; mean, 3 days) and chronic (>4 weeks) benign, one-kidney, one clip (1K1C) hypertension. Thus, we measured vascular responses in vasodilated (nitroprusside or adenosine), vascularly isolated, innervated hindlimb vascular beds of chloralose-anesthetized 1K1C rats perfused with their own blood at 1 ml/min. Complete norepinephrine dose-response curves in 8 rats with chronic and 28 with early 1K1C hypertension, compared with appropriate normotensive control rats, showed unchanged thresholds and ED\(_{50}\) values. Magnitude of ouabain-induced leftward shifts of the norepinephrine dose-response curve in 18 rats with chronic and 21 with early 1K1C hypertension, compared with appropriate normotensive control rats, was unchanged. Blockade of neural uptake of norepinephrine by desimipramine (10\(^{-7}\) M) in 81K1C rats did not alter these results.

These findings provide no evidence in this form and these stages of hypertension that humoral ouabainlike inhibitors of the Na\(^+\)-K\(^+\) pump evoke physiologically significant inotropic effects in arterioles in vivo. It is possible, however, that induction of vascular Na\(^+\)-K\(^+\)-adenosine triphosphatase by circulating inhibitors modified the vascular responses to norepinephrine and ouabain in these rats. (Hypertension 10 [Suppl I]: I-95-I-100, 1987)

KEY WORDS • Na\(^+\)-K\(^+\)-ATPase • ouabain • arteriolar responses • norepinephrine • vascular resistance • Goldblatt hypertension

SEVERAL laboratories have provided evidence for a humoral inhibitor, or inhibitors, of Na\(^+\),K\(^+\)–adenosine triphosphatase (ATPase) in hypertension, especially in volume-dependent, salt-sensitive, or low renin forms.\(^1\)–\(^8\) It has been suggested that such a substance(s) inhibits the Na\(^+\)-K\(^+\) pump in vascular tissue, contracting the vascular muscle and increasing sensitivity to norepinephrine, thereby elevating resistance, and hence, causing and sustaining hypertension.\(^1\)–\(^7\)\(^,\)\(^8\)

Several critical links in this hypothesis have not been established. For example, one laboratory\(^5\) reported that plasma from hypertensive rats produced acute pump inhibition when assayed in arteries excised from normotensive rats. This, however, does not establish that hypertensive plasma would have the same effect if assayed on hypertensive arteries that had already been continuously exposed to such plasma factors, as would be the case in vivo. Furthermore, many other laboratories, including ours, found increases, rather than decreases, in pump activity of arterial tissue excised from rats with chronic hypertension.\(^9\)–\(^11\) We suggested that induction of additional Na\(^+\)-K\(^+\) pump molecules by the circulating inhibitor(s) might account for the increased pumping.\(^9\) In this regard, Kim et al.\(^12\) reported that chronic exposure of chick myocardial cells in culture to ouabain induced Na\(^+\)-K\(^+\)-ATPase.

It has also not been established that the circulating inhibitor(s) has chronic inotropic effects on vessels in vivo. Furthermore, it is considerably debated whether functional vasoconstriction, such as that postulated to be produced by circulating inhibitor(s) or other agents, accounts for the elevated resistance in chronic stages of hypertension. Folkow's group\(^13\) provided considerable evidence incriminating structural vascular changes alone.

To further investigate some of these links in the hypothesis, we studied in vivo arteriolar responses to norepinephrine and ouabain in rats with one-kidney, one clip (1K1C) hypertension, perfusing the hindlimb bed with the rat's own blood, presumably containing the pump inhibitor substance(s). The present paper compiles and summarizes results previously published separately.\(^14\),\(^15\)
Methods

Male Sprague-Dawley rats weighing 150 to 190 g (Zivic-Miller Laboratories, Allison Park, PA, USA) underwent the following procedures: 1) unilateral nephrectomy and partial constriction of the contralateral (left) renal artery with a 0.38-mm (inside diameter) silver clip (chronic 1K1C hypertensive rats); 2) unilateral nephrectomy and sham clipping (chronic 1K normotensive control rats); 3) unilateral nephrectomy and, 2 weeks later, partial constriction of the contralateral renal artery with a 0.46-mm (inside diameter) clip (early 1K1C rats); or 4) unilateral nephrectomy and, 2 weeks later, sham constriction of the contralateral renal artery (early 1K rats).

Rats were maintained on standard rat chow (Na⁺, 0.39%; K⁺, 0.96%). Chronic 1K1C rats drank either water or saline (1% NaCl), chronic 1K rats drank water, early 1K1C rats drank saline, and early 1K rats drank either water or saline. Results in comparable rats drinking saline did not significantly differ from those in rats drinking water, so data were pooled. We measured body weight and tail systolic blood pressure (in unanesthetized rats by the tail cuff method: Natsume Rat Tail Manometer System, Model No. KN-0090, Natsume Company, Tokyo, Japan) twice weekly in chronic 1K1C and 1K rats and daily in early 1K1C and 1K rats. Early 1K1C rats usually became hypertensive within 24 to 48 hours (systolic pressure > 140 mm Hg). After 4 weeks of hypertension in the chronic 1K1C rats, 2 to 7 days of hypertension in the early 1K1C rats, and at an equal time interval in the appropriate normotensive control (1K) rats, we performed hindlimb perfusion studies.

The rats were anesthetized with chloralose, 100 mg/kg i.v. The hindlimb was vascularly isolated, but sciatic and femoral nerves and the femoral vein were left intact. The limb was pump-perfused by techniques previously described. The pump was filled (about 4 ml) with heparinized blood from a donor rat of the same strain and type. Then blood from the carotid artery of each heparinized rat was pumped at a constant rate of 1 ml/min into the femoral artery of its isolated hindlimb, with venous return to the rat’s body through its femoral vein. The perfusion was continued for 30 minutes to establish a steady state, at which time resting limb vascular resistance was calculated. Perfusion pressure was monitored by a Statham P23Gb pressure transducer (Hato Rey, Puerto Rico) and a Hewlett-Packard recorder (Palo Alto, CA, USA).

In most rats we maintained the hindlimb at maximal vasodilation during response studies to minimize differences in initial vascular resistance between hypertensive and normotensive rats. Such differences have important and complex influences on vascular responses to norepinephrine, influences that make data interpretation difficult. In some rats we used adenosine injections and then infusions to evoke and maintain maximal vasodilation. In these rats we injected supramaximal doses of adenosine into the pump tubing upstream from the pump to achieve maximal vasodilation. We then infused adenosine into the upstream pump tubing at 0.085 mg in 0.011 ml saline/min, achieving levels of 0.085 mg/ml limb blood, levels that maintained maximal hindlimb vasodilation. In other rats we injected and then infused sodium nitroprusside, 0.015 mg in 0.005 ml saline/min, into the upstream pump tubing to achieve 0.015 mg/ml limb blood, a rate that maintained a maximal hindlimb vasodilation that was comparable to what we had achieved with adenosine.

With infusions of adenosine or nitroprusside continuing, we assayed complete dose-response relationships to norepinephrine (levaterenol bitartrate, Sigma) over the range of 0.0005 to 128 μg (each dose uniformly injected as 0.005 ml upstream to the pump). We gave the next injection when limb resistance had returned to baseline (maximal vasodilation) levels or after 15 minutes, whichever occurred first. We calculated responses as peak increase in limb resistance (perfusion pressure minus pressure gradient across the cannula, divided by blood flow normalized to limb weight) over resistance at maximal vasodilation. We compared responses and ED₅₀ values between hypertensive and normotensive rats by unpaired, two-tailed Student’s t test, with the null hypothesis rejected at p < 0.05.

In other rats with limb resistance also maintained at maximal vasodilation with adenosine or sodium nitroprusside, we studied the shift in the norepinephrine dose-response curve induced by exogenous ouabain. In these rats we first measured perfusion pressure responses to three levels of norepinephrine before ouabain. Then we infused ouabain (Sigma) into the upstream pump tubing at a rate (5 mM at 0.005 ml/min) calculated to elevate limb blood concentrations to 2.5 x 10⁻³ M. This is a level that we have found inhibits ouabain-sensitive ⁸⁶Rb⁺ uptake by rat tail arteries in vitro by 40 to 50% (unpublished observations) and that increases norepinephrine responses in the perfused rat hindlimb. Higher doses of ouabain further enhance limb vascular responses to norepinephrine. We started the ouabain infusion 3 minutes before norepinephrine was reinjected and continued it for an additional 3 minutes, by which time the maximal response (peak height) to the norepinephrine injection had occurred.

In additional early 1K1C and 1K rats receiving adenosine infusions we also blocked limb neural uptake of norepinephrine by infusion into the pump tubing of desmipramine (DMI, Merrell Dow), achieving concentrations of 10⁻⁷ M in limb blood. Preliminary studies in six rats indicated that this concentration maximally increased limb norepinephrine responses.

For data analysis we compared the height of peak responses (occurring prior to any systemic effects of the norepinephrine injections) of 1K1C rats with 1K rats using the unpaired, two-tailed Student’s t test.

We measured hematocrit and plasma creatinine in each rat. We necropsied each rat to verify clip placement and health. We weighed the cardiac ventricles and hindlimb, and expressed limb resistance in terms of hindlimb wet weight.
**Results**

Significant hypertension developed in the clipped rats (mean carotid arterial pressure ± SEM: 128 ± 2, 108 ± 3, 152 ± 4, and 106 ± 4 mm Hg in early 1K1C, early 1K, chronic 1K1C, and chronic 1K rats, respectively). Hypertension was accompanied by significant increases in the ratio of ventricular weight to body weight (p < 0.001), small increases in creatinine in chronic 1K1C rats only, and slight increases in hematocrit in early 1K1C rats only. Average duration of hypertension in early 1K1C rats was about 3 days. Most experiments in early 1K1C and 1K rats were performed within 5 days, and all within 10 days, of clipping or sham clipping. In early 1K1C rats, calculated limb resting resistance was elevated by 45% (p < 0.001). Rises in resting resistance of 97% (p < 0.001) occurred in chronic 1K1C rats. With maximal vasodilation with nitroprusside, resistance in early 1K1C rats remained elevated by 15% (p < 0.02), and resistance in chronic 1K1C rats remained elevated by 34% (p < 0.001).

**Responses to Norepinephrine**

Complete limb norepinephrine dose-response curves are presented in Figure 1. Responses in the presence of nitroprusside in eight chronic 1K1C hypertensive and five chronic 1K normotensive control rats are presented in Figure 1. The norepinephrine dose-response curves are steeper and higher in the hypertensive rats. However, there is no change in threshold or in ED$_{50}$ (5.50 ± 0.30 vs 6.00 ± 0.30 μg norepinephrine in 1K1C and 1K rats, respectively; p > 0.3). Figure 2 presents similar data in the case of nine early 1K1C and 15 1K rats in the presence of nitroprusside.

In these rats with early disease, curves in hypertensive and normotensive rats are virtually indistinguishable. This was also true of curves in 10 early 1K1C and 10 early 1K rats vasodilated with adenosine (data not presented). In contrast, in Figure 3 curves in nine early 1K1C and 13 early 1K rats in the absence of vasodilators demonstrate slight but significant leftward shifts of the curves in 1K1C rats. The ED$_{50}$ values were lower (p < 0.02) in the hypertensive rats (0.50 ± 0.07 μg norepinephrine in 1K1C vs 0.95 ± 0.14 μg in 1K rats). At the dose levels at which differences between responses were observed, however, there were significant correlations (p < 0.01) between magnitude of responses and the initial resistance levels in these rats. This correlation suggests that the greater responses in the 1K1C rats may be explained on the basis of their higher levels of resting resistance, and points out the importance of minimizing differences in initial resistance when comparing responses in hypertensive and normotensive animals, as we did by infusing nitroprusside or adenosine.

**Responses to Ouabain and Norepinephrine**

Significant leftward shifts in the exponential portion of the norepinephrine dose-response curve were induced by infusion of ouabain (Figures 4 and 5). Figure 4, representing responses in chronic 1K1C and 1K rats, illustrates that ouabain-induced shifts tended to be greater, if anything, in hypertensive than in normotensive rats. Figure 5 represents ouabain-induced shifts in 11 early hypertensive and 12 normotensive rats vasodilated with nitroprusside, again illustrating no statistically significant differences in ouabain responses in the two groups. Time-control experiments in 12 other
early 1K1C and 1K rats (not shown) also indicated no differences between hypertensive and normotensive rats. Results in 16 early 1K and 16 early 1K1C rats vasodilated with adenosine (not shown) were similar.

The ouabain infusions affected both vascular smooth muscle and sympathetic neurons in the hindlimbs. It is possible that the similar ouabain responses in hypertensive and normotensive rats reflected equal but opposite myogenic and neurogenic effects. For this reason, in a final study in 12 early 1K1C and 12 early 1K rats, we blocked neural uptake of norepinephrine by infusing desipramine into the pump tubing along with adenosine to achieve limb blood concentrations of $10^{-7}$ M. Comparison of responses (data not presented) again revealed no significant differences in either time-control studies or in the magnitude of the ouabain-induced shifts.

**Discussion**

The results of the present studies provide little evidence in early stages and no evidence in chronic stages of 1K1C hypertension in rats for significant increases in sensitivity of arterioles to norepinephrine. Other investigators reported similar in vivo findings. The present investigation also indicates that the arteriolar response to ouabain is unaltered in these hypertensive rats. These rats had a form of hypertension considered to be volume-expanded or volume-dependent. Responses in rats drinking saline did not differ from those drinking water. We and other laboratories have reported evidence for plasma-borne inhibitors of Na\(^{+}\),K\(^{-}\)-ATPase in this form of hypertension. Thus our findings suggest that the limb arterioles are not responding in the anticipated manner to the circulating inhibitor(s). With significant levels of endogenous ouabainlike material, we would have expected increased sensitivity to norepinephrine and attenuated leftward shifts of the norepinephrine dose-response curve induced by exogenous ouabain.

Our results have several possible explanations. First, it is possible that our methods obscured real differences in responses between normotensive and hypertensive rats. We feel this is unlikely, because, using identical techniques, including vasodilator infusions, we were readily able to detect denervation hypersensitivity in perfused rat hindlimbs. In addition, Göthberg et al. also maintained vascular beds at maximal or near-maximal dilation and were able to detect significant augmentation of ouabain-induced leftward shifts of norepinephrine dose-response curves.
in spontaneously hypertensive rats (SHR), suggesting to them increased activity levels of Na⁺,K⁺-ATPase in vascular tissues.

A second possible explanation is that smooth muscle hypersensitivity in the hypertensive rats was obscured by equal and opposite increases in limb neural uptake of norepinephrine. We believe we excluded this explanation with our experiments blocking neuronal uptake with desimipramine. Using similar blockade, Webb et al. ⁹ were able to demonstrate smooth muscle hypersensitivity in SHR.

Third, it is possible that there is no circulating endogenous digitalislike material or that levels are too low to evoke physiologically significant effects. Clearly then, the hypothesis, at least with regard to 1K1C hypertension in rats, is in error and the detected inhibitor artifactual or physiologically insignificant.

We favor the alternative explanation that, with time, the circulating ouabainlike inhibitor(s) in the hypertensive rats induces Na⁺,K⁺-ATPase in vascular tissues, just as long-term (days) exposure to ouabain induces enzyme in cultured myocardial cells ¹² or guinea pig hearts in vivo. ²⁰ Thus, at the time of our studies, a normal or near-normal number of unoccupied pump sites accounted for the normal responses to norepinephrine and were available for interaction with exogenous ouabain. In this regard, Kim et al. ¹² found that the induced Na⁺,K⁺-ATPase modified the inotropic responses of myocardial cells. We propose that induced Na⁺,K⁺-ATPase in the vascular tissues of our hypertensive rats modified (normalized) their responses to norepinephrine and to ouabain.

If our explanation is correct, it is certainly questionable whether a link exists between the detected circulating ouabainlike materials(s) and arteriolar contraction and elevated resistance in chronic 1K1C hypertension in rats. However, it is certainly possible that the detected circulating substance(s) raises arteriolar resistance in hypertension by noninotropic mechanisms, for example, by producing vascular "waterlogging" ²¹, ²² or by enhancing vascular smooth muscle growth, ²³ or both.

References
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