Vascular Responses to Ouabain and Norepinephrine in Low and Normal Renin Hypertension

ROBERTO PEDRINELLI, STEFANO TADDEI, LINDA GRAZIADEI, AND ANTONIO SALVETTI

SUMMARY A circulating Na+, K+-ATPase inhibitor may cause arterial hypertension in patients with suppressed plasma renin activity, either directly or by sensitizing peripheral vessels to α-adrenergic stimulation. This hypothesis was tested by evaluating forearm arteriolar (plethysmographic technique) response to exogenous α-adrenergic stimulation by a 2-minute intra-arterial infusion of norepinephrine (0.1 µg/dl tissue per minute) and to Na+, K+-ATPase inhibition by sequential 20-minute intra-arterial infusions of ouabain (0.36 and 0.72 µg/dl tissue per minute). Two groups of hypertensive subjects with suppressed plasma renin activity, either essential or secondary to aldosterone excess, were compared with age-matched and sex-matched hypertensive subjects with normal plasma renin activity (n = 7 per group). No significant differences in forearm vascular response to norepinephrine were found among the three groups. Ouabain caused a highly significant, dose-related increment in forearm vascular resistance that was not accompanied by changes in the contralateral limb or systemic blood pressure. No significant interindividual differences in vascular responsiveness to ouabain were found. The individual increments in forearm vascular resistance during ouabain administration were unrelated to basal values or to plasma aldosterone, norepinephrine, or potassium concentrations. These data are not consistent with the hypothesis that suppressed basal Na+, K+-ATPase activity is primarily a characteristic of hypertensive patients with unresponsive plasma renin activity. Overall, these results cast doubts on the possibility of linking the development of human low renin hypertension to an endogenous Na+, K+-ATPase inhibitor. (Hypertension 8: 786-792, 1986)

KEY WORDS • Na+, K+-ATPase • renin • aldosterone • α-adrenergic responsiveness • ouabain • hypertension

THE hypothesis that a circulating Na+, K+-ATPase (Na+–K+ pump) inhibiting factor might cause human hypertension has received considerable attention.1-3 This putative factor might cause vasoconstriction either directly or by sensitizing the peripheral vasculature to α-adrenergic stimuli. This latter mechanism may act by causing depolarization, which consequently lowers the threshold of the contraction range in response to vasoconstrictor agents. More recently, evidence has been gathered to suggest that suppressed peripheral plasma renin activity (PRA) values, either in essential hypertension or secondary to aldosterone excess, may be a marker for the presence of such a factor in humans (see Reference 2 for a review).4-7

The present study was designed to further assess this possibility. Our specific aims were to test the forearm vascular response to α-adrenergic stimulation, using norepinephrine (NE) infused intra-arterially in the forearm vasculature, and Na+, K+-ATPase activity is primarily a characteristic of hypertensive patients with unresponsive plasma renin activity. Overall, these results cast doubts on the possibility of linking the development of human low renin hypertension to an endogenous Na+, K+-ATPase inhibitor. (Hypertension 8: 786-792, 1986)
because of postural uptake in plasma aldosterone, bilateral adrenal uptake of [131I]iodocholesterol on the adrenal scintiscan, and negative adrenal computed tomographic scan. Since age might influence the amount of a circulating Na+ K+ ATPase inhibitor, these subjects were matched for age (± 2 years) and sex with two groups of subjects classified according to their renin-sodium index 10 as having low renin or normal renin, mild to moderate essential hypertension. The subjects in these two groups had been screened during a previous admission to the clinic and then recruited for the present study. According to institutional guidelines, all subjects gave their informed consent after a detailed description of the study was offered. Exclusion criteria included the presence of malignant hypertension, renal damage, or coexisting systemic disease.

All subjects discontinued any medication and were then seen at weekly intervals during the 3 weeks preceding admission to the clinic. All had outpatient blood pressure readings higher than 140/90 mm Hg. Each triplet of subjects (i.e., the subject with PA and his or her matched pair with either LREH or NREH) was studied in the same week as clinic inpatients. A normocaloric diet with constant electrolyte intake (sodium, 80–100 mmol/day; potassium chloride, 60–80 mmol/day) was maintained throughout the study, and compliance was assessed daily by measuring urinary sodium and potassium excretion. The PRA; plasma NE and aldosterone (with subjects in the standing position at 1000); plasma sodium and potassium; daily urinary excretion of aldosterone, sodium, and potassium; and creatinine clearance were obtained on either the fourth or the fifth day of admission.

That same day, the effect of NE and ouabain on forearm blood flow (FBF) was studied. After a 20-minute equilibration period, during which saline was infused (0.206 ml/min) to obtain reproducible basal FBF rates, a 2-minute NE infusion was started at a rate of 0.1 μg/dl of forearm tissue per minute. After a second 20-minute saline infusion (or longer if needed) to reattain basal values, ouabain was administered at two different infusion rates (20 minutes each), 0.36 and 0.72 μg/dl of forearm tissue per minute, a dosage range already shown to be effective in humans. Preliminary dose titration studies had shown that, for either NE or ouabain, these infusion periods were sufficient to attain a response plateau. We also found that ouabain infusion rates greater than those eventually used caused further vasoconstriction, but with associated changes in systemic blood pressure and heart rate. On the other hand, lower amounts of the drug caused vasoconstriction that was often difficult to distinguish from the inherent variability of the method. Overall, these data convinced us that the ouabain infusion rates finally chosen covered a major part of the vasoconstrictor dose-response curve to ouabain in forearm vasculature.

The FBF studies were conducted in the afternoon with the subjects seated comfortably in a warm room (22°–25°C).

With the subject under light local anesthesia (2% xylocaine), a polyethylene cannula (Abbo cath 18–20 gauge; Abbot, Sligo, Ireland) was inserted into the left brachial artery and connected through stopcocks to a pressure transducer (MS20, Electromedics, Englewood, CO, USA) for systemic mean blood pressure (calculated as 1/3 pulse pressure + diastolic pressure) monitoring (VSM1, Physio Control, Redmond, WA, USA) and to a Harvard infusion pump (Series 944A, Ealing, South Natick, MA, USA) for intra-arterial drug administration. A strain-gauge plethysmograph (Loosco, G.L. Loos, Amsterdam, Holland) was used to measure changes in the volume of both forearms (left, cannulated; right, control). In our laboratory, strips are made of Silastic tubing of 0.4 mm inside diameter and 0.8 mm outside diameter filled with mercury. The gauge was applied to the subject’s arm 5 to 6 cm distal to the elbow at a tension sufficient to keep the gauge in the same position throughout the experiment. The subject’s forearms were kept on a table, slightly flexed and inclined at about a 45-degree angle, with the wrists and hands supported by sand bags. One minute before FBF determination, a pneumatic pediatric cuff was placed around both wrists and inflated to suprasystolic arterial blood pressure to exclude the vascular region of the hand. A second cuff was placed proximal to the plethysmograph and automatically inflated to a pressure of 40 mm Hg to allow FBF measurement according to the venous occlusion method described by Whitney. Preliminary experiments showed that brachial artery cannulation per se caused no FBF changes.

Determination of forearm volume was performed according to the water displacement method. Drug infusion rates were normalized for 1 dl of tissue by adjusting the speed of infusion to the desired infusion rates. The PRA and plasma and urinary aldosterone were measured by radioimmunoassay, plasma NE by high-pressure liquid chromatography, and plasma and urinary electrolytes by flame photometry using lithium as an internal standard.

For the NE experiments, FBF was recorded continuously throughout a 2-minute infusion period. The lowest FBF value recorded during the last 30 seconds of infusion was chosen as a measure of the effect of the agonist on the vasculature. For the studies with ouabain, three FBF determinations were obtained every 3 minutes and averaged. All the tracings were read by a single observer (S.T.) and had a within-observer variability of 3.5% (variation coefficient; 9 blind readings of the same tracing). Under these experimental conditions, repeated FBF measurements (during a 1-hour period) in a separate group of seven subjects gave a 15.5 ± 6.1 (SD)% variation coefficient in the absence of any intervention.

Forearm vascular resistance (FVR), expressed in arbitrary units resulting from the ratio of mean arterial pressure to the corresponding FBF, was used to evaluate the effect of pharmacological manipulations on forearm arterioles. Statistical analysis was performed through nonparametric methods by using either the
Wilcoxon test for paired comparison or analysis of variance according to Kruskall-Wallis. Areas under the curve (FVR vs time) were approximated through the trapezoidal rule. Correlation coefficients were calculated according to the least-squares method. A p level less than 0.05 was chosen as significant. Results were expressed as means ± SEM unless otherwise specified.

The (-)NE (Levophed) and ouabain (Ouabaine Arnaud) were obtained from commercially available sources and diluted in fresh solutions to the desired concentrations by adding normal saline. Syringes containing NE were wrapped in aluminum foil to prevent deterioration due to light exposure.

**Results**

At the time of the hemodynamic study, all subjects had reached a constant sodium excretion rate (Table 1). As expected, subjects with PA had higher urinary and plasma aldosterone and lower plasma potassium levels than did the other two groups. No statistical difference in PRA was present between the PA and LREH groups; both groups differed from the NREH group in this regard. Mean arterial pressure, recorded indirectly or directly, was comparable between the PA and LREH groups and was higher than that seen in the NREH group (p<0.01). Mean FBF tended to be lower in the LREH group, which resulted in significantly higher FVR values than in the PA and NREH groups (p<0.01; see Table 1).

Infusion of NE caused a highly significant increment in FVR in all subjects that was independent of basal values (r = 0.08) and was not accompanied by systemic blood pressure or contralateral FVR changes. The increase in FVR (ratio of the infused to the control forearm) during NE infusion tended to be lower in the PA group, higher in the NREH group, and intermediate in the LREH group. Overall, however, no statistically significant differences were found between groups (Figure 1). The individual increments in FVR during the NE infusion were unrelated to basal FVR (r = 0.10) or to plasma potassium (r = 0.28, n = 21), urinary aldosterone (r = -0.41, n = 21), and plasma NE (r = 0.39, n = 21) levels. Similar results were found when each group was analyzed separately.

No significant changes in systemic arterial pressure or contralateral FVR occurred during ouabain infusion. Although FVR increased consistently in all subjects (within the first 5 to 10 minutes) in the infused limb, there was no correlation with basal values. On the average, FVR values at the end of infusion at the

<table>
<thead>
<tr>
<th>Variable</th>
<th>PA (n = 7)</th>
<th>LREH (n = 7)</th>
<th>NREH (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>43.6 ± 2.3</td>
<td>42.7 ± 2.6</td>
<td>42.2 ± 2.6</td>
</tr>
<tr>
<td><strong>Sex (M/F)</strong></td>
<td>4/3</td>
<td>4/3</td>
<td>4/3</td>
</tr>
<tr>
<td><strong>Median duration of hypertension (yr)</strong></td>
<td>5.0</td>
<td>7.5</td>
<td>6.8</td>
</tr>
<tr>
<td><strong>PRA (ng ANG I/ml/hr)</strong></td>
<td>0.05 ± 0.01</td>
<td>0.25 ± 0.07</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALDO (ng/ml)</td>
<td>65.9 ± 10.3*</td>
<td>27.2 ± 5.9</td>
<td>38.9 ± 6.9</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>129.6 ± 9.3</td>
<td>149.6 ± 20.9</td>
<td>170.2 ± 15.2</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>145.0 ± 0.4</td>
<td>143.6 ± 1.0</td>
<td>142.7 ± 0.8</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>2.9 ± 0.2*</td>
<td>3.7 ± 0.07</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td><strong>Urinary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALDO (µg/24 hr)</td>
<td>60.7 ± 11.5*</td>
<td>15.6 ± 6.4</td>
<td>14.8 ± 6.9</td>
</tr>
<tr>
<td>Na (mmol/24 hr)</td>
<td>85.0 ± 6.7</td>
<td>96.1 ± 13.4</td>
<td>97.0 ± 8.1</td>
</tr>
<tr>
<td>K (mmol/24 hr)</td>
<td>57.0 ± 6.4</td>
<td>46.3 ± 6.0</td>
<td>46.9 ± 4.6</td>
</tr>
<tr>
<td>Ccr (ml/min)</td>
<td>122.8 ± 9.3</td>
<td>123.8 ± 9.9</td>
<td>115.5 ± 8.8</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>121.1 ± 5.1</td>
<td>125.6 ± 4.8</td>
<td>110.6 ± 5.0†</td>
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<tr>
<td>MIBP (mm Hg)</td>
<td>119.1 ± 8.1</td>
<td>130.6 ± 7.4</td>
<td>106.4 ± 5.0†</td>
</tr>
<tr>
<td>Forearm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BF (ml/min·dl)</td>
<td>4.5 ± 0.4</td>
<td>3.6 ± 0.3</td>
<td>4.0 ± 0.0</td>
</tr>
<tr>
<td>VR (ml·mm Hg/min·dl)</td>
<td>29.5 ± 3.7</td>
<td>39.0 ± 2.8†</td>
<td>28.2 ± 2.0</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>1080.1 ± 111.9</td>
<td>992.9 ± 81.3</td>
<td>1145.7 ± 81</td>
</tr>
</tbody>
</table>

Most values are means ± SEM.

PA = primary hyperaldosteronism; LREH = low renin essential hypertension; NREH = normal renin essential hypertension; PRA = plasma renin activity; ANG I = angiotensin I; ALDO = aldosterone; NE = norepinephrine; Ccr = creatinine clearance; MBP = mean blood pressure; MIBP = mean intra-arterial blood pressure; BF = blood flow; VR = vascular resistance.

* p < 0.001, compared with LREH and NREH groups.
† p < 0.01, compared with LREH group.
‡ p < 0.01, compared with PA and NREH groups.
rate of 0.72 μg/dl of forearm tissue per minute were significantly greater (p<0.01) than those recorded at the lower infusion rate (Figure 2). No significant differences in the response of FVR (infused/control forearm) to ouabain were found among the three groups, analyzed either as values at each time point (Figure 3) or as integrated area-under-the-curve values (PA group, 1.35 ± 0.1; LREH group, 1.34 ± 0.1; NREH group, 1.36 ± 0.09 ml-mm Hg/min-dl for 40 minutes). Changes in FVR (expressed as the ratio of infused to control area-under-the-curve values), analyzed individually or as a group, were unrelated to plasma potassium (r = —0.10, n = 21), sodium (r = 0.10, n = 21), and plasma NE (r = 0.32, n = 21) levels.

Discussion

In 1981, MacGregor et al. found that the ability of plasma to stimulate glucose-6-phosphate dehydrogenase (an index of the plasma Na⁺, K⁺-ATPase inhibiting activity) was significantly greater in subjects with low PRA than in those with normal PRA. The same group of investigators also reported that another index of Na⁺-K⁺ pump activity, the leukocyte ouabain-sensitive Na⁺ efflux rate, was lower in subjects whose PRA did not rise normally during sodium restriction, as compared with normally responsive hypertensive subjects. Similarly, Burris et al. reported that ouabain-sensitive Rb uptake in the rat tail artery, another index of Na⁺-K⁺ pump activity, was depressed by the addition of plasma from hypertensive subjects to the medium. This effect was more pronounced when plasma from subjects with LREH rather than NREH was used. Overall, these results suggested the presence of a circulating Na⁺, K⁺-ATPase inhibiting factor in hypertensive subjects with unresponsive PRA. The present study further examined this possibility in three prospectively selected groups of subjects, matched for age and sex and stratified according to renin and aldosterone levels. Of the other biological parameters measured, the only significant between-group difference was in blood pressure (see Table 1), particularly between the LREH and the NREH groups, the latter of which had significantly lower values. This finding is in agreement with the inverse relationship between blood pressure and peripheral renin documented in large-scale studies.

In addition to their direct vasoconstrictor activity, digitalis glycosides sensitize vascular smooth muscle to sympathetic neural stimulation and exogenous NE in vitro, probably as a consequence of their membrane depolarizing effect. Since digoxin administration increases pressor responsiveness to exogenous NE in normal humans, a circulating Na⁺, K⁺-ATPase inhibiting factor might have comparable effects on vascular responsiveness to adrenergic stimulation. Circumstantial evidence in favor of this possibility has come from the observation that human plasma from hypertensive subjects, some of whom had low peripheral renin values, sensitized isolated human and animal vascular tissues to the vasoconstrictor effect of exogenous NE. In our study, exogenous NE was infused into the forearm vasculature at a rate that had enabled other investigators to detect a higher degree of responsiveness in otherwise unselected hypertensive and normotensive subjects. In spite of the apparent sensitivity of the technique, we were unable to show significant between-group differences in arteriolar responsiveness to exogenous α-adrenergic stimulation. The data do not allow detailed speculation about possible mechanisms that might play some role in individual variations, but if resting blood pressure is, on the whole, a fair reflection of the average vascular pressure load, then vascular hypertrophy should have been more marked in the LREH group than in the NREH group. Thus, if vascular restructuring was present and contributing to arteriolar reactivity, the responsiveness to a given dose of NE in the PA and LREH groups should have been even lower than that in the NREH group, since vascular response was comparable in all groups. Therefore, even if a Na⁺, K⁺-ATPase inhibitor circulated in greater amounts in the LREH subjects, it could not have been associated with increased vascular responsiveness to exogenous NE, as might be theorized from some results reported in the literature.

In this study, the vasoconstrictor effect of ouabain on forearm arterioles was considered a physiologically relevant indicator of basal Na⁺-K⁺ pump activity, an assumption validated by previous human studies that also showed the independence of the vascular effect of ouabain from the α-adrenergic system, at least in humans. Others, using the same conceptual ap-
Figure 2. Effect on forearm vascular resistance (FVR, expressed as ml·mm Hg/min·dl) of two increasing infusion rates of ouabain (O) injected into the brachial artery of subjects with primary hyperaldosteronism (top panel), low renin essential hypertension (middle panel), or normal renin essential hypertension (bottom panel). The FVR in the contralateral forearm (●) during the infusion is shown for comparison. Values are means ± SEM (n = 7 per group). See text for the statistical evaluation of the data.

approach, reported contrasting data, suggesting either depressed or increased Na⁺-K⁺ activity in hypertensive subjects. However, those authors did not select their subjects according to peripheral renin values and, more importantly, did not include subjects with PA (i.e., the closest clinical approximation to the low renin, volume-expanded animal models from which was elaborated the concept of the involvement of endogenous Na⁺-K⁺ pump inhibitors in hypertension; see Reference 2 for a review).
Our data clearly confirm that digitalis glycosides exert a direct vasoconstrictor effect on human FVR, and thus strongly suggest that Na\(^+\), K\(^+\)-ATPase is functionally active in humans and can cause vasoconstriction when acutely inhibited. However, we did not find any between-group differences in the response to two increasing levels of ouabain. This finding is in variance with the assumption of a reduced pharmacological effect of ouabain, if greater amounts of a circulating digitalislike factor decreased the number of available receptors for ouabain itself. Therefore, these data are not consistent with the hypothesis that vascular Na\(^+\), K\(^+\)-ATPase is depressed to a greater extent in LREH. Our results, however, are relevant from another, rather divergent perspective.

Experimental studies in rats have shown that the permeability of the vascular smooth muscle membrane to sodium ions is increased by aldosterone, and this may account to some extent for the vascular hyperactivity observed in deoxycorticosterone acetate-treated or aldosterone-treated animals. Accordingly, the vascular Na\(^+\)-K\(^+\) pump should compensate for the increased passive sodium leak by increasing its activity, as suggested by in vitro experiments. Yet our subjects with hypermineralocorticoidism were not characterized by any evident change in vascular response to Na\(^+\), K\(^+\)-ATPase inhibition (or to \(\alpha\)-adrenergic stimulation) in comparison to subjects with normal aldosterone levels, irrespective of their renin levels. We cannot, however, exclude the possibility that a complex interplay between low plasma potassium levels, high aldosterone levels, and volume expansion might have obscured the results.

In conclusion, we cannot negate, on the basis of our results, the possibility that a circulating digoxinlike factor might be elevated in distinct hypertensive subjects and linked to the development of high blood pressure, as the data of either Hamlyn et al. or Devyuck et al. suggest. Neither can we exclude the possibility that positive results might have been obtained under different experimental conditions, such as sodium loading. As far as this last point is concerned, however, we must note that, despite a relatively low sodium intake (around 100 mmol/day), our subjects (especially those with LREH) had high blood pressure. This finding suggests that a putative hypertensinogenic factor, if present, must have been active even at the time of the study. Altogether, our data cast doubt on the possibility of linking the development of human low renin hypertension, as a whole, with disturbances in the activity of vascular Na\(^+\), K\(^+\)-ATPase.

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