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Vascular Smooth Muscle Membrane Potential in Rats with Early and Chronic One-Kidney, One-Clip Hypertension (42288)

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Abstract. It has been suggested that the cell membrane of vascular smooth muscle in one-kidney, one-clip hypertension, and other forms of volume-dependent, low-renin hypertension, is partially depolarized due to the effects of a circulating ouabain-like factor, and that this depolarization is an important mechanism of the hypertension. Levels of circulating ouabain-like factors in early stages of volume-dependent hypertension are reported equal to, or greater than, those in chronic hypertension. Therefore, we measured intracellular membrane potentials (E_m) *in vitro* (37°C, physiological salt solution) in vascular smooth muscle of the caudal artery from normotensive control rats (1K) and rats in the early and chronic stages of one-kidney, one-clip hypertension (1K1C). In 20 chronic 1K1C (4–6 weeks of systolic pressure >140 mm Hg) the resting E_m's (M ± SEM) were −46.7 ± 0.7 mV, compared to −50.9 ± 0.6 for 20 1K ($P < 0.01$). The ΔE_m due to 1 m*M* ouabain was attenuated in 10 1K1C compared to 11 1K (+5.4 ± 0.9 and +10.0 ± 0.7 mV, respectively; $P < 0.01$). The E_m's of the two groups after ouabain were the same. In contrast, in 16 early 1K1C rats (<7 days hypertension, average 3 days) compared to 15 appropriate 1K, there were no significant alterations in resting E_m (−50.1 ± 0.4 mV, compared to −50.5 ± 0.5, respectively) and there were no differences in ouabain response. These results suggest a temporal dissociation between levels of humoral inhibitors and depolarization, and between depolarization and hypertension, and thus fail to support the hypotheses that there are causal relationships between these variables in volume-dependent, low-renin hypertension.

In 1972, it was first proposed (1) that in hypertension a defect in the operation of the vascular smooth muscle electrogenic Na^+–K^+ pump decreases membrane potential in arteriolar smooth muscle. This partial depolarization impairs vascular relaxation and hence, elevates blood pressure. Considerable evidence has appeared supporting elements of this hypothesis and extending it to venous and cardiac muscle (2–9). Reduced numbers of sarcolemmal pump molecules have been found in spontaneously hypertensive rats (9, 10). Other observations suggest a circulating inhibitor of the membrane Na^+–K^+ pump in forms of hypertension considered "volume-dependent and low-renin" (2–8, 11). Partial depolarization of vascular smooth muscle from rats with chronic one-kidney, one-clip hypertension has been reported by investigators in one laboratory (12), which is compatible with the hypothesis.

On the other hand, Blaustein (13) argues that chronic suppression of the membrane Na^+–K^+ pump by, for example, a circulating inhibitor, would increase cell sodium content. This, in turn, would restore pump activity, and hence, membrane potential, toward normal levels. According to this view, one would not expect to observe chronic membrane depolarization under these circumstances.

Furthermore, the major component of cell membrane potential is contributed by the passive diffusion potential, reflecting the transmembrane distribution of ions and membrane ion permeabilities (14). Jones (15) has provided evidence for changes in membrane ion permeability in forms of genetic and acquired hypertensions, that under some circumstances, reduce membrane potential in vascular smooth muscle cells (16).

Additionally, we (17) and others (Songu-Mize *et al.*, personal communication) found *in vitro* evidence for increases, rather than decreases, in ouabain-sensitive $^{86}Rb^+$ uptake by vascular smooth muscle of tail arteries from rats with chronic one-kidney, one-clip hypertension. This finding suggests that their membrane depolarization may not reflect decreased activity of the Na^+–K^+ pump.

Finally, we have provided evidence that

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levels of circulating Na^+–K^+ pump inhibitors in the early stages of one-kidney, one-clip hypertension are at least as high as levels in chronic stages (18). Similar findings in other forms of volume-dependent, low-renin hypertension have been reported by other laboratories (19) (V. Buckalew, personal communication).

Thus, in the present investigation we measured membrane potential in vascular smooth muscle of rats with both early and chronic stages of one-kidney, one-clip hypertension. If the partial depolarization were attributable to the circulating pump inhibitors, one would expect membrane effects early in hypertension, persisting or even diminishing as the hypertension became chronic. Similarly, if the hypertension is attributable to membrane depolarization, such depolarization should be present in the early, as well as the chronic, stages of the hypertension.

Materials and Methods. To create chronic one-kidney, one-clip hypertension, male Sprague–Dawley rats weighing 150–190 g (Zivic Miller Laboratories, Inc., Allison Park, Pa.) underwent unilateral nephrectomy and partial constriction of the contralateral (left) renal artery with an i.d. 0.38 mm silver clip (1K1C) or unilateral nephrectomy and sham-clipping (1K, normotensive control rats). Rats were maintained on standard rat chow (Na^+ = 0.39%; K^+ = 0.96%) and water or saline for drinking *ad libitum* thereafter. Body weights and conscious tail systolic blood pressures by the tail cuff method (Natsume Tail Manometer System, Model KN-0090) were measured in all rats weekly. After 4–6 weeks of sustained hypertension (systolic pressure >140 mm Hg) in the chronic hypertensive rats, and at an equal time interval in the control rats, we obtained arterial tissue for measurement of membrane potentials.

To create early one-kidney, one-clip hypertension, the rats underwent only unilateral (right) nephrectomy at 150–190 g. Two to three weeks later, with rats at body weights of 300–320 g, we clipped (1K1C) or sham-clipped (1K) the left renal artery with a larger silver clip, i.d. 0.46 mm. 1K1C rats received saline and 1K rats received saline or water for drinking *ad libitum* thereafter and were maintained on the rat chow described above. We measured body weights and conscious tail systolic blood pressures daily. Early 1K1C rats usually became hypertensive within 24–48 hr (systolic pressure >140 mm Hg). After 2–7 days of sustained hypertension (but less than 10 days after clipping) in the hypertensive group, and at an equal time interval in the control rats, we obtained arterial tissue for measurement of membrane potentials.

For each experiment the rat was anesthetized (pentobarbital, 75 mg/kg ip) and the caudal artery removed. A 1-cm segment was placed in ice-cold physiological salt solution (composition in m*M*: 146 Na^+, 4.8 K^+, 2 Ca^{2+}, 1 Mg^{2+}, 127 Cl^-, 27 HCO_3^-, and 25 glucose). The caudal artery segment was pinned to a Sylgard layer on the bottom of the *in vitro* chamber. While viewing the artery segment through a dissecting microscope (10 to 40×), we carefully cleaned the adventitial side. The suffusion fluid (also physiological salt solution) was maintained at 37°C and flowed by gravity at a rate of 2–3 ml/min. Solutions were oxygenated with 95% O_2–5% CO_2 and buffered at pH 7.4. Solutions were prepared on the day of the experiment with highest grade chemicals available.

The vascular smooth muscle cells in the artery were impaled with microelectrodes from the adventitial side. The microelectrodes were pulled from capillary tubes (1.2 mm o.d.; WPI, H1B120P) with a Model P-77 Brown-Flaming micropipet puller. The microelectrodes were filled with 3 *M* KCl and the average tip resistance was >60 MΩ. The membrane potential was recorded with a WPI model M701 microprobe amplifier, a storage oscilloscope and a Gould strip chart recorder. A WPI A.B.M. audio monitor was used to detect change in voltage as the microelectrode tip entered a cell. The chamber and micromanipulator were on a vibration-free table and the microscope and other accessories sat on the floor of an independently supported Faraday cage. Criteria for an acceptable puncture were (i) a sharp increase in the E_m as the tip entered the cell; (ii) an E_m of −40 mV or greater; (iii) an increase in the microelectrode tip resistance of no more than 30 MΩ as the cell was punctured; (iv) a stable E_m for at least 30 seconds; and (v) a return of E_m to control values as the tip was withdrawn from the cell. During a 1-hour control period the chamber containing the artery was suffused with the physiological salt

solution. After the control period, resting E_m was measured in several cells of the tail artery. Then, to assess the E_m response, suffusion was changed to physiological salt solution containing ouabain (Sigma) 1 mM final concentration. If possible a stable micropuncture was maintained during a change in test conditions; when a single micropuncture could not be maintained, multiple punctures were made. In each rat, values of E_m's in several cells for each treatment were averaged before data were further processed. Data analysis was by two-tailed Student's t test, either unpaired or paired as appropriate. Null hypothesis was rejected at $P \leqslant 0.05$.

Results. All rats appeared healthy and of stable weight or gaining weight at the time of study. Mean values (±SEM) for final body weight, ventricular weight/body weight, average conscious tail systolic blood pressure, duration of hypertension, hematocrit, and plasma creatinine, Na^+, and K^+ of 1K1C and 1K rats are presented in Table I. Significant hypertension developed in the clipped rats accompanied by increases in ventricular weight and ventricular weight/body weight, greater in the rats with chronic hypertension. Average duration of hypertension in early 1K1C was 3 days. There were no significant differences between early hypertensive and normotensive rats in body weight, hematocrit, or plasma Na^+, K^+ or creatinine. Findings were similar in the chronic 1K1C, except for decreases in body weight. In no hypertensive rat reported, early or chronic, was plasma creatinine greater than 1.0 mg%.

Within each group values in rats drinking saline were similar to those in rats drinking water, so data were pooled.

The resting membrane potentials (E_m) in chronic and early rats and the effects of ouabain are presented in Table II and Fig. 1. A sample record of the ΔE_m due to ouabain is shown in Fig. 1. This figure demonstrates reversibility and provides an estimate of time required for ouabain action (onset approximately 3 min, stable effect within 10 min at the suffusion rate used). In other experiments it was noted that the effects were not completely reversible if the tissue was exposed to ouabain for >30 min.

In the absence of ouabain, 407 cells were successfully punctured in 40 chronic 1K1C and 1K rats, and 326 cells were punctured in 31 early 1K and 1K1C rats (Table II). In chronic 1K1C rats, resting E_m was reduced by 8% ($P < 0.01$). In contrast, in early 1K1C rats no significant alterations in E_m were detected.

TABLE I. SUMMARY OF ANIMALS USED (MEAN ± SEM)

	Chronic (>4 weeks)				Early (2–7 days)			
	n	1K	n	1K1C	n	1K	n	1K1C
Body wt, g	20	329 ± 16	20	292 ± 10[b]	15	362 ± 9	16	354 ± 12
Ventricular wt, g	20	0.869 ± 0.044	20	1.184 ± 0.064[b]	15	0.903 ± 0.084	16	1.165 ± 0.034[b]
Ventricular wt/body wt, g/g × 10^5	20	256 ± 4	20	438 ± 15[b]	15	277 ± 9	16	313 ± 17[a]
Systolic blood pressure, mm Hg	20	114.7 ± 1.2	20	171.1 ± 9.9[b]	15	114.8 ± 1.4	16	147.8 ± 2.3[b]
Duration of hypertension, days			20	24 ± 1			16	2.8 ± 0.2
Hematocrit, vol%	20	40.4 ± 1.2	20	40.7 ± 0.9	15	39.9 ± 0.5	16	39.8 ± 0.5
Plasma Na^+, meq/liter	19	149.8 ± 0.7	19	151.0 ± 1.5	7	150.1 ± 2.4	6	148.0 ± 0.8
Plasma K^+, meq/liter	19	3.76 ± 0.19	19	3.80 ± 0.23	7	4.63 ± 0.49	6	4.63 ± 0.48
Plasma creatinine, mg%	19	0.76 ± 0.07	19	0.89 ± 0.06	7	0.67 ± 0.11	6	0.80 ± 0.04

Note. For comparison between 1K and 1K1C, [a]$P < 0.05$; [b]$P < 0.01$.

TABLE II. RESTING E_m (mV) AND EFFECT OF 1 m*M* OUABAIN (MEAN ± SEM)

	Chronic (>4 weeks)				Early (2–7 days)			
	1K		1K1C		1K		1K1C	
	n	E_m	*n*	E_m	*n*	E_m	*n*	E_m
Control	20/229	−50.9 ± 0.6	20/178	−46.7 ± 0.7[a]	15/164	−50.5 ± 0.5	16/162	−50.1 ± 0.4
Ouabain	11/24	−41.2 ± 0.6	10/18	−41.4 ± 1.2	6/10	−42.0 ± 1.6	6/9	−40.8 ± 1.0
ΔE_m due to ouabain	11/24	+10.0 ± 0.7	10/18	+5.4 ± 0.9[a]	6/10	+8.0 ± 0.9	6/9	+9.4 ± 1.1

Note. *n* = number of rats/number of punctures.
[a] $P < 0.01$, for comparison between 1K and 1K1C.

In response to ouabain, depolarization occurred in all punctured cells. Within each group this depolarization was significant ($P < 0.01$). Stable E_m during exposure to ouabain did not differ between hypertensive and normotensive rats. However, as Table II indicates, in 10 rats with chronic 1K1C hypertension the ΔE_m due to ouabain was reduced by 46% ($P < 0.01$) as compared to 11 1K rats. In contrast, in the early rats, ouabain evoked equal degrees of depolarization in hypertensives and normotensives.

Discussion. It has been suggested (12, 20) that membrane depolarization observed in vascular smooth muscle of rats with chronic one-kidney, one-clip hypertension is attributable to inhibition of the Na^+–K^+ pump by a circulating ouabain-like factor. To support a cause–effect argument one must demonstrate a temporal relationship between variables. In this regard, elevated levels of the humoral factor have been observed in early stages of volume-dependent, low-renin hypertension in rats (19) (V. Buckalew, personal communication), diminishing as the hypertension becomes chronic. In a recent study of rats with 1K1C hypertension prepared identically to those reported here, Magargal and Overbeck (18) (and additional unpublished observations), assayed plasma using cultured aortic

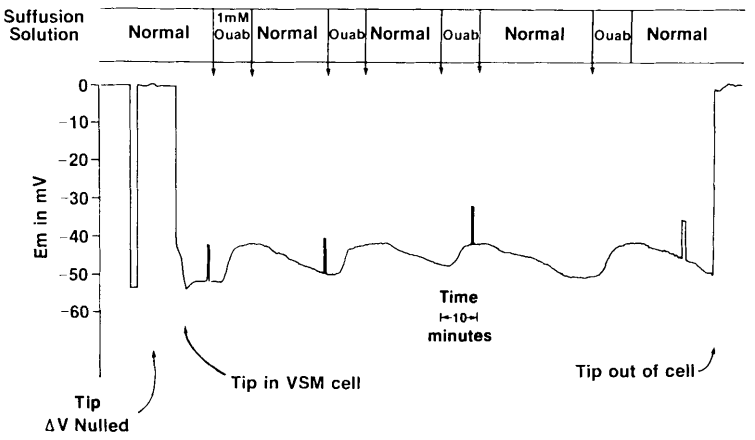

FIG. 1. Effects of ouabain on E_m. With the tip of the microelectrode in the bath, the E_m was zero. The large downward deflection in response to current injection allowed estimate of the tip resistance. The small upward deflection in E_m after balancing was also due to current injection. The sudden hyperpolarization was due to the electrode tip entering a cell. The subsequent upward spikes were response to current injection to assess cell membrane resistance. "Normal" indicates suffusion with physiological salt solution; "Ouab" indicates 10-min suffusion with physiological salt solution containing 1 m*M* ouabain. Temperature, 37°C; chamber volume, 2.5 ml; suffusion rate, 5 ml/min.

smooth muscle cells from normal rats. Cells in the presence of plasma from rats with early or chronic hypertension had 5–10% reductions ($P < 0.01$) in ouabain-sensitive $^{86}Rb^+$ uptake, when compared to cells incubated in normotensive plasma. There were trends suggesting higher levels of inhibitory factors in plasma from the early 1K1C rats. Thus, one would expect to observe magnitudes of depolarization in early 1K1C hypertension at least as great as those in chronic hypertension, if the membrane depolarization is attributable to this humoral inhibitory factor.

In rats with chronic 1K1C hypertension in the present study we observed the same levels of vascular smooth muscle membrane depolarization that have been reported (12). Additionally, the membrane depolarization evoked by ouabain was attenuated, as has been observed (12). In contrast, in rats with early 1K1C hypertension, we found no evidence for membrane depolarization or for attenuated ouabain responses, although measurements were made under identical conditions. It is possible that in early hypertension the circulating inhibitor is bound less firmly to the pump molecule, dissociating during the dissection and preincubation periods. However, there is no evidence in support of this explanation. Incubation of the tail arteries in the rat's plasma, rather than in salt solution, might have revealed depolarization in early 1K1C, and certainly, *in vitro* studies may not reflect *in vivo* conditions. However depolarization was present in chronic 1K1C arteries even when incubated *in vitro* in salt solution under identical conditions.

Thus, our results provide evidence that there may be a temporal dissociation between levels of humoral inhibitors and depolarization in volume-dependent, low-renin hypertension. Our results fail to support the hypothesis that there is a causal relationship between humoral inhibitor and membrane depolarization.

The major contribution to membrane potential is the passive diffusion potential, reflecting transmembrane distribution of ions and membrane ion permeabilities. Thus, we attempted to measure membrane ion resistance, but the variance of our results was too great to allow us to draw convincing conclusions. Future studies using patch-clamp procedures (21) may provide direct information about the status of membrane ion permeability in the vascular smooth muscle cells of 1K1C hypertensive rats. Nevertheless, we suggest that the best explanation for the reduced membrane potential in vascular smooth muscle in chronic 1K1C hypertension in rats is an increased membrane ion permeability of unknown mechanism (15, 16). We suggest that differences in levels of baseline E_m may have accounted for the lesser effect of ouabain in chronic 1K1C. Alternatively, ouabain may have had different effects on membrane Ca^{2+} permeability in normotensive and hypertensive rats (22).

Finally, our data also suggest a temporal dissociation between the membrane depolarization and the hypertension in 1K1C rats. The early 1K1C rats had significant elevations of blood pressure but no membrane depolarization. Although again *in vitro* studies may not reflect *in vivo* conditions, our findings do not appear to support the hypothesis (1, 12, 20) that membrane depolarization in this form of hypertension contracts the vascular smooth muscle, producing hypertension.

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