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Angiotensin-Converting Enzyme Inhibition During Development Alters Calcium Regulation in Adult Hypertensive Rats[1]

OREN TRAUB[2] and R. CLINTON WEBB
Department of Physiology, The University of Michigan, Ann Arbor, Michigan
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ABSTRACT
Studies have shown that angiotensin-converting enzyme (ACE) inhibitor treatment in young genetically hypertensive rats prevents the full expression of blood pressure and vascular abnormalities in the adult. This model provides unique conditions with which to study the pathogenesis of altered Ca^{++} regulation. Normotensive (WKY) rats and stroke-prone spontaneously hypertensive rats (SHRSP) received at 6 to 10 weeks of age either ACE inhibitor (ramipril), hydralazine/hydrochlorothiazide or no treatment. At 17 weeks of age, rats were anesthetized, and vascular tissue was excised. Thoracic aorta challenged with 20 mM caffeine in Ca^{++}-free buffer produced a phasic contractile response. The magnitude of this phasic response was used as a measure of Ca^{++} released from intracellular stores; a direct correlation between this phasic response and systolic blood pressure was observed. A concentration-response curve to Bay K8644 was performed on carotid arteries; a direct correlation of force development to Bay K8644 and systolic blood pressure was observed. All WKY groups showed lower blood pressure and force development in response to Bay K8644 than did SHRSP. Treatment with ramipril reduced blood pressure and force development in response to Bay K8644 in adult SHRSP, although not to levels of WKY rats, whereas WKY rats were unaffected by treatment. These data support the hypothesis that contractile responses to Bay K8644 in carotid arteries and caffeine in aorta parallel changes in systolic blood pressure. We conclude that alteration of Ca^{++} regulation in hypertension is directly related to elevated blood pressure and mediated by an angiotensin II-sensitive mechanism during development.

Many studies have documented pathological alterations in cellular Ca^{++} handling in patients with essential hypertension and have confirmed the effectiveness of Ca^{++} channel antagonists in reducing systemic blood pressure (Ram, 1985; Schwartz *et al.*, 1984; Scriabine *et al.*, 1988; Urthaler, 1986; Vanhoutte, 1985). Furthermore, investigators report that small changes in intracellular Ca^{++} concentrations can cause large changes in vascular tone (Morgan, 1987) and therefore may be responsible for the enhanced vascular reactivity found in the smooth muscle of hypertensive animals. Increased permeability to Ca^{++} has been demonstrated in the plasma membrane of arteries from hypertensive animals, possibly due to a decreased membrane-stabilizing effect (Furspan *et al.*, 1986; Furspan and Bohr, 1988; Rinaldi and Bohr, 1988) and a greater activity of L-type Ca^{++} channels (Hermsmeyer *et al.*, 1989, 1990). It has also been proposed that alterations in the storage or release of Ca^{++} from intracellular stores (SR) may contribute to the increased vascular reactivity in hypertension; studies report greater release of Ca^{++} from the SR of vascular smooth muscle in both genetic (Hermsmeyer *et al.*, 1989b) and experimental models (Perry and Webb, 1991) of hypertension.

Some controversy exists, however, as to whether this alteration in Ca^{++} metabolism is the cause or the result of increased vascular tone and reactivity. Recently, an aspect of the genetically hypertensive animal has been reported that provides a unique opportunity to explore the question of the pathogenesis of hypertension and its relation to altered Ca^{++} handling. Several investigators have shown that SHR treated with an ACE inhibitor during a "critical period" of development (2 to 10 weeks of life) fail to have elevated arterial blood pressure to the same magnitude as that observed in untreated SHR or SHR treated with the ACE inhibitor during a noncritical period (Giudicelli *et al.*, 1980; Harrap, 1992; Harrap *et al.*, 1990; Lee *et al.*, 1991; Morton *et al.*, 1992). This effect has since been confirmed in the SHRSP. The SHRSP treated with ACE inhibitors have blood pressures that are significantly lower in adulthood than their untreated SHRSP counterparts, yet they retain the same genetic predisposition for hypertension.

This modified model of hypertension in which the full expres-

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ABBREVIATONS: ACE, angiotensin-converting enzyme; hydral/HCTZ, hydralazine/hydrochlorothiazide; PSS, physiological salt solution; SR, sarcoplasmic reticulum; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats; 5-HT, serotonin; WKY, Wistar-Kyoto.

sion of high blood pressure is prevented may be particularly useful in separating vascular alterations that are primary to hypertension from those that are secondary to development of high blood pressure. Through the use of this modified genetic model, we previously determined that increased adrenergic sensitivity is not related to blood pressure and may be a primary cause of hypertension. The present study focuses on altered Ca^{++} handling in hypertension and whether alteration in Ca^{++} handling or sensitivity is a primary or secondary factor of hypertension. We used this modified genetic model of hypertension to consider Ca^{++} handling at the level of the plasma membrane as well as the SR and determine its relationship to blood pressure. The hypothesis tested was that increased responsiveness to Ca^{++} channel agonists is reduced in adult SHRSP treated with ramipril during development and that reversal of the vascular abnormalities in hypertension is independent of the blood pressure-lowering effect of ACE inhibitors. A non-angiotensin-dependent antihypertensive treatment was used to test whether long-term changes in vascular reactivity are dependent on blood pressure lowering during development or whether changes were specific to ACE inhibition.

Materials and Methods

Animals. Male SHRSP and WKY rats were obtained from a colony maintained in the Department of Anatomy and Cell Biology at the University of Michigan (Ann Arbor, MI). All rats were housed in a light-cycled, temperature-controlled room with ad libitum access to food and water. At all times excluding the 6- to 10-week age period, rats received tap water and standard laboratory chow (Purina Mills Co., Richmond, IN). Systolic blood pressure was measured weekly by an indirect tail cuff (pneumatic transducer) method in conscious, restrained rats. Blood pressure values were averaged in sets of two consecutive weeks, and blood pressure data are reported in 2-week intervals. Three experimental treatment regimens were included in this protocol: untreated (WKY, n = 8; SHRSP, n = 8), ACE inhibitor treated (WKY, n = 8; SHRSP, n = 8), and diuretic/vasodilator treated (WKY, n = 6; SHRSP, n = 6). One diuretic/vasodilator-treated SHRSP and one untreated WKY rat did not survive the study and were excluded from the analysis. Rats in the diuretic/vasodilator group received hydralazine (200 mg/l) and hydrochlorothiazide (200 mg/l) in their drinking water during the treatment period. Based on an intake of 20 ml of water per day, rats received 4 mg/day of each drug. All other rats received tap water. Rats in the ACE inhibitor-treated group were given ramipril in their food (0.0025% ramipril by weight) from 6 to 10 weeks of age. Measured dosages of ramipril were 2.94 ± 0.16 mg/kg/day for WKY rats and 3.23 ± 0.18 mg/kg/day for SHRSP (P > .05). All other rats received control food.

Experimental protocol. At 17 weeks of age, rats were anesthetized with sodium pentobarbital (50 mg/kg) and decapitated. Thoracic aortas and carotid arteries were harvested and placed into cold PSS with a composition (in mM) of NaCl, 130; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_2 \cdot 7H_2O$, 1.17; $NaHCO_3$, 14.9; dextrose, 5.5; $CaNa_2$-EDTA, 0.026 and $CaCl_2$, 1.6 for ~20-hr storage at 3.5°C. Preliminary experiments showed that no significant change in contractile responsiveness accompanied this overnight storage. Fatty and connective tissue was removed from the thoracic aortas and carotid arteries, and the vessels were cut into helical strips (2 mm × 15 mm for aorta, 0.7 mm × 10 mm for carotid arteries). The endothelium was removed by gentle rubbing of the intima with a cotton swab. Vascular strips were mounted on stainless-steel tissue hangers and suspended in a muscle bath containing PSS maintained at 37°C and aerated with 95% O_2-5% CO_2. Calcium-free buffer contained 1.0 mM EGTA and no added calcium. Strips were allowed to equilibrate for 90 min with a passive force of 1.5 × g and 0.6 × g for aortas and carotid arteries, respectively. Drugs were added directly to the bath, and isometric contractile force was measured using Grass FT.03 force transducers in Quincy, MA and recorded on a Grass Polygraph. Between responses, agonists were rinsed from the tissue bath, and strips were allowed to recover to base-line values. In thoracic aorta, contractile responses to 5-HT and caffeine in Ca^{++}-free buffer after loading of a cellular Ca^{++} store were measured by a method similar to that described by Karaki *et al.* (1979). In addition, cumulative concentration-response curves to Bay K8644, a calcium channel agonist, were generated using carotid arteries.

Drugs. Bay K8644, caffeine, hydralazine hydrochloride, hydrochlorothiazide, and serotonin creatine sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). Ramipril was the gift of Upjohn Co. (Kalamazoo, MI). Laboratory chow containing 0.0025% ramipril by weight was prepared for this study by Purina Mills Co. Serotonin creatine sulfate was prepared in 0.1% ascorbate solution in deionized water, and Bay K8644 was prepared in ethanol. All other drugs were prepared and diluted in deionized water. Other chemicals used in this study were of reagent grade.

Statistical analysis. Data are expressed as mean ± S.E.M. Unpaired Student's t tests were used to determine statistical significance between SHRSP and WKY rats and within groups. A value of $P < .05$ was considered significant. The Bonferroni correction was applied to adjust for multiple comparisons. Correlation of systolic blood pressure with vascular responsiveness was calculated using linear regression analysis.

Results

Blood pressures at 16 weeks of age were as follows (mm Hg): untreated WKY, 131 ± 4; hydral/HCTZ-treated WKY, 132 ± 3; ramipril-treated WKY, 131 ± 3; untreated SHRSP, 217 ± 4; hydral/HCTZ-treated SHRSP, 198 ± 6 and ramipril-treated SHRSP, 168 ± 2. Fig. 1 illustrates the effect of early brief antihypertensive treatment on the development of blood pressure. Ramipril treatment from 6 to 10 weeks of age in the SHRSP significantly reduced blood pressures at 16 weeks of age from untreated levels (fig. 1B), although not to WKY levels, and WKY rats were unaffected by early treatment (fig. 1A). SHRSP treated with hydral/HCTZ from 6 to 10 weeks of age also showed significantly lower blood pressures at 16 weeks of age but not to the magnitude of the ramipril-treated SHRSP (fig. 1B). The values reported in fig. 1 are a summary of blood pressure data presented in an earlier study from our laboratory.

Fig. 2 illustrates contractile force development in response to caffeine in SHRSP and WKY aorta. Aortas were stimulated with 5-HT (3 μM), and the contraction was allowed to reach a plateau. Ca^{++}-free buffer was then introduced into the muscle bath, and the strips were allowed to equilibrate for 10 min; this period corresponds to the Ca^{++} store-depletion period. Subsequently, Ca^{++}-free buffer was replaced by buffer containing 1.6 mM Ca^{++}, and the strips were allowed to equilibrate for 10 min; this period corresponds to the Ca^{++} store-loading period. After this period, Ca^{++}-free buffer was reintroduced into the bath, and aortas were allowed to equilibrate for 30 sec before 20 mM caffeine was introduced. The resulting phasic response to caffeine in Ca^{++}-free buffer reflected Ca^{++} released from intracellular stores.

The absolute magnitude of phasic contractile responses to caffeine was significantly greater in aortas from untreated SHRSP than in arteries from untreated WKY rats (fig. 3A). This relationship held when the phasic contractile response was also expressed as a percent of the initial response to 5-HT. (Maximal responses to 3 μM 5-HT in milligrams of force were as follows: untreated WKY, 426 ± 72; hydral/HCT-treated WKY, 670 ± 54; ramipril-treated WKY, 653 ± 57; untreated

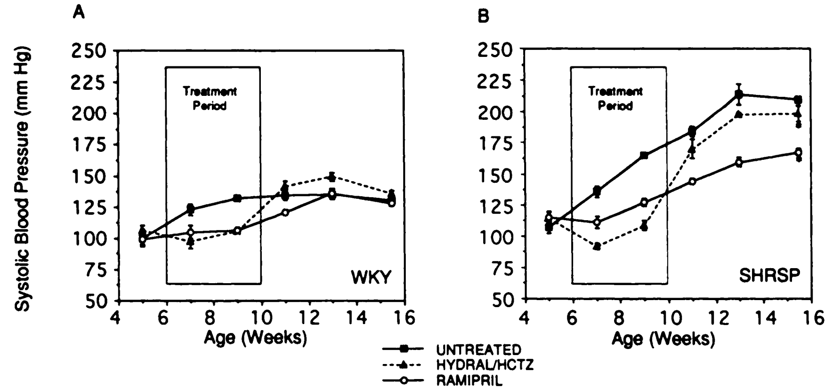

Fig. 1. Antihypertensive regimen effects on blood pressure. A, Line graph showing indirect systolic blood pressure of WKY rats from 5 to 16 weeks of age receiving no treatment ($n = 7$), hydral/HCTZ treatment ($n = 6$) or ramipril treatment ($n = 8$). B, Line graph showing indirect systolic blood pressure of SHRSP from 5 to 16 weeks of age receiving no treatment ($n = 8$), hydral/HCTZ treatment ($n = 5$) or ramipril treatment ($n = 8$). Blood pressures were averaged over 2-week intervals and plotted at the midpoint of corresponding ages. All data are reported as mean ± S.E.M. values. *$P < .01$ compared with untreated group by t test in week 16.5 of blood pressure measurements. The values reported are a summary of blood pressure data presented in an earlier study from our laboratory.

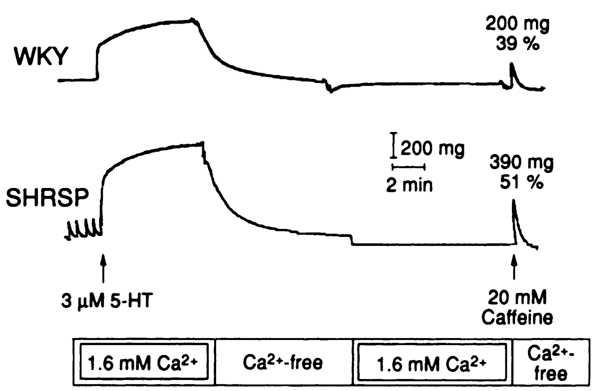

Fig. 2. Cellular Ca++ stores in rat aorta. Aortas from SHRSP and WKY rats were stimulated with 5-HT (3 μM) and allowed to reach a plateau. Ca++-free buffer was then introduced into the muscle bath, and strips were allowed to equilibrate for 10 min. After this depletion period, 1.6 mM Ca++ buffer was placed into the muscle bath, and strips were allowed to equilibrate for 10 min. After this loading period, Ca++-free buffer was introduced into the bath and allowed to equilibrate for 30 sec before 20 mM caffeine was added. Phasic contractile responsiveness was greater in aortas from SHRSP compared with aortas from WKY rats, even when contractile response is expressed as a percentage of the initial response to 5-HT.

SHRSP, 895 ± 47; hydral/HCT-treated SHRSP, 892 ± 147 and ramipril-treated SHRSP, 863 ± 51). Neither antihypertensive treatment had a significant effect on phasic contractile responsiveness to caffeine in the WKY rats. All SHRSP showed significantly greater magnitudes of phasic contractile force than the WKY rats. Although treatment with hydral/HCTZ in SHRSP did not significantly change phasic contractile responsiveness to caffeine from untreated SHRSP levels, treatment with ramipril significantly decreased contractile responsiveness to caffeine from untreated SHRSP levels, although not to WKY levels. Fig. 3B shows the relationship between terminal systolic blood pressure and phasic contractile responsiveness to caffeine. A positive correlation between blood pressure and phasic contractile response was observed when linear regression was performed. None of the animal groups were statistically different from the regression line.

Fig. 4 illustrates contractile force development to Bay K8644 in SHRSP and WKY carotid arteries. Carotid arteries were first depolarized with 100 mM KCl to determine maximal force development. No significant difference was observed between the WKY and SHRSP in any of the treatment groups (~260 mg developed force to 100 mM KCl). The KCl was washed out, and the vessels were allowed to relax to base line before beginning a cumulative concentration-response curve to Bay K8644. Force development from base line was observed in the untreated SHRSP at 30 nM Bay K8644 and increased with higher concentrations of agonist. Carotid arteries from untreated WKY animals, however, showed only phasic spikes of force development at 300 nM and did not generally show force development from base line at any concentration. Several WKY rats did show force development from base line as shown in fig. 5.

Fig. 5A illustrates cumulative concentration-response curves to Bay K8644 in treated and untreated animals in milligrams of force. At 1 μM Bay K8644, neither antihypertensive treatment had a significant effect on force development in the WKY rats. All SHRSP showed significantly greater magnitudes of force development in response to 1 μM Bay K8644 than the WKY rats. Although treatment with hydral/HCTZ in SHRSP did not significantly change force development from untreated SHRSP levels, treatment with ramipril significantly decreased force development from untreated SHRSP levels. Fig. 5B shows the relationship between terminal systolic blood pressure and force development in response to 1 μM Bay K8644. A positive correlation between blood pressure and force development in response to 1 μM Bay K8644 was observed when a linear regression is performed. None of the animal groups were statistically different from the regression line.

Discussion

A principal finding of this study was that blood pressure levels in adult SHRSP can be reduced if the rats are treated with ramipril at 6 to 10 weeks of age. The observations on blood pressure confirm the early work of Harrap *et al.* (1990), which has been extended in studies by Lee *et al.* (1991), and Morton *et al.* (1992). Harrap *et al.* (1990) proposed the existence of an angiotensin II-sensitive genetic program mediating blood pressure that can be altered by blocking the production of angiotensin II during a critical developmental period. We have used this "modified model" of hypertension to explore vascular

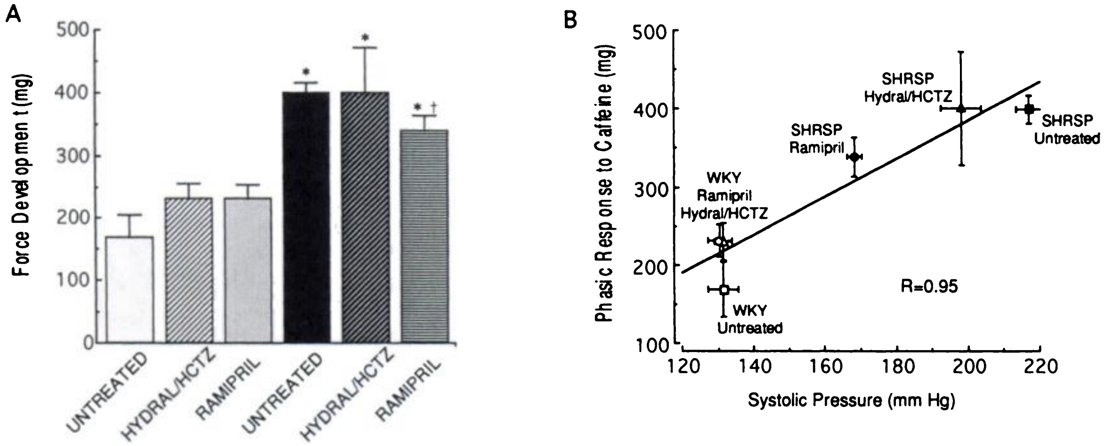

Fig. 3. A, Development of phasic contractile force to caffeine in Ca^{++}-free buffer in aortic strips from SHRSP and WKY (treated and untreated animals). Contractile force in all SHRSP groups was significantly greater than force development in untreated WKY (*$P < .05$). Contractile responses in aortas from ramipril-treated SHRSP group were significantly less than the untreated-SHRSP response (†$P < .05$). B, Correlation between phasic contractile force development in all animal groups and terminal systolic blood pressure. A linear regression was performed using all group points, and it was determined that a positive correlation exists between blood pressure and phasic contractile response to caffeine. None of the groups are significantly different from the regression line. All data are expressed as mean ± S.E.M. values for untreated WKY (n = 7), hydral/HCTZ WKY (n = 6), ramipril-treated WKY (n = 8), untreated SHRSP (n = 8), hydral/HCTZ-treated SHRSP (n = 5), and ramipril-treated SHRSP (n = 8).

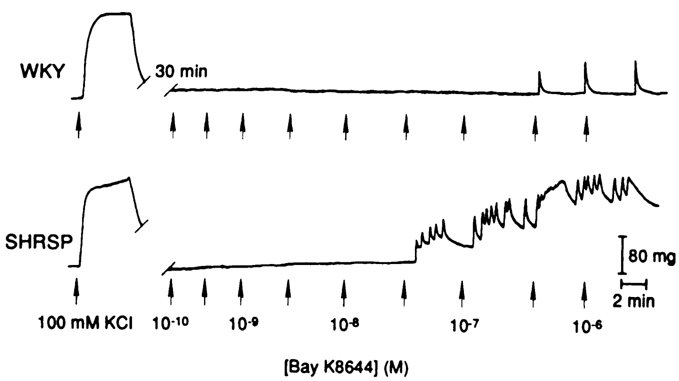

Fig. 4. Responsiveness to Ca^{++} channel agonist Bay K8644 in the carotid arteries. Carotid arteries from SHRSP and WKY rats were challenged with 100 mM KCl. Maximal force development in the carotid arteries from SHRSP was not significantly different from that in WKY rats. Arteries were allowed to recover, and a cumulative concentration-response curve to Bay K8644 was performed. Carotid arteries from SHRSP were more sensitive to the agonist, and force development was observed in these vessels. Force development was not observed in WKY arteries, and responsiveness is characterized by phasic contractile spikes in these vessels. Other WKY vessels did or did not show force development from base line (see fig. 5).

changes that accompany an alteration in expression of blood pressure in SHRSP treated with ramipril during development.

The present study confirms previous work demonstrating that Ca^{++} handling at the level of the plasma membrane (Furspan *et al.*, 1986; Furspan and Bohr, 1988; Hermsmeyer *et al.*, 1989, 1990; Rinaldi and Bohr, 1988) and the SR (Hermsmeyer *et al.*, 1989b; Perry and Webb, 1991) is altered in hypertension. Carotid arteries from SHRSP were more sensitive to the Ca^{++} channel agonist Bay K8644 and developed significantly greater magnitudes of force compared with carotid arteries from WKY rats at all concentrations. Furthermore, aortas from SHRSP showed greater magnitudes of phasic contractile force in response to caffeine in Ca^{++}-free PSS than did aortas from WKY rats.

The Ca^{++} channel agonists Bay K8644 and caffeine activate different Ca^{++} channels and cause contraction *via* different cellular mechanisms. Bay K8644 opens voltage-sensitive Ca^{++} channels in the plasma membrane (Kanmura *et al.*, 1984). Extracellular Ca^{++} subsequently enters the cell and causes contraction in vascular smooth muscle. In contrast, caffeine enters the cell and acts directly to open ryanodine-sensitive Ca^{++}-efflux channels that line the SR, releasing Ca^{++} from intracellular stores and causing a phasic contraction (Rousseau and Meissner, 1989).

The data presented here extend previous observations by showing that contractile responses to 1 μM Bay K8644 or 20 mM caffeine (in Ca^{++}-free buffer) in carotid arteries and aortas from ramipril-treated SHRSP were intermediate between those of untreated SHRSP and WKY rats. Furthermore, the change in vascular responsiveness correlated with terminal systolic blood pressures as seen by linear regression analysis. A change in vascular responsiveness was not seen, however, in SHRSP treated with hydral/HCTZ. Thus, the developmental pathway that affects blood pressure level and/or contractile responsiveness to Bay K8644 and caffeine is related to an angiotensin II-sensitive mechanism during development.

The present study provides no evidence that the long-term changes in vascular reactivity are due to the specific capacity of ramipril to block angiotensin II generation rather than other effects of the ACE inhibitor. The rationale for the use of this label derives from other studies involving brief angiotensin II-blocking treatments during development. Both captopril and perindopril have been shown to have long-term effects of blood pressure similar to those of ramipril and therefore, it is assumed, similar changes in vascular reactivity (Harrap *et al.*, 1990; Lee *et al.*, 1991). Furthermore, the angiotensin II receptor antagonist losartan has been shown to have similar effects on long-term blood pressure when administered during development (Morton *et al.*, 1992). In the landmark study by Harrap *et al.* (1990), this long-term effect on blood pressure was not observed when angiotensin II was coadministered with ACE inhibitors during development. Therefore, it seems likely that these long-term changes attributed to ramipril treatment during development are due to the compound's ability to block generation of angiotensin II.

We proposed that reduced adrenergic sensitivity and blood pressure in SHRSP treated with ramipril were associated with

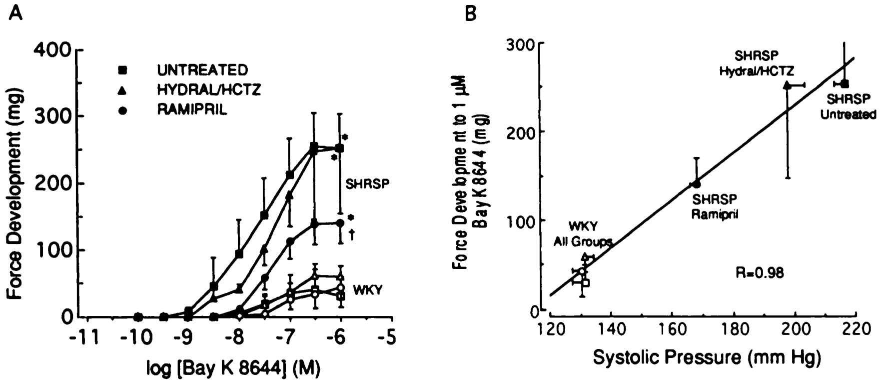

Fig. 5. A, Development of contractile force to Bay K8644 in the carotid arteries from SHRSP and WKY (treated and untreated animals). Contractile force in all SHRSP groups was significantly greater than force development in untreated WKY (*P < .05). Contractile responses in the carotid arteries from ramipril-treated SHRSP group to 1 μM Bay K8644 were significantly less than that of untreated-SHRSP response (†P < .05). B, Correlation between force development in response to 1 μM Bay K8644 in all animal groups and terminal systolic blood pressure. A linear regression was performed using all group points, and it was determined that a positive correlation exists between blood pressure and force development in response to 1 μM Bay K8644. None of the groups are significantly different from the regression line. All data are expressed as mean ± S.E.M. values for untreated WKY (n = 7), hydral/HCTZ-treated WKY, (n = 6), ramipril-treated WKY (n = 8), untreated SHRSP (n = 8), hydral/HCTZ-treated SHRSP (n = 5) and ramipril-treated SHRSP (n = 8).

an angiotensin II-sensitive developmental program and therefore was a primary contributing factor in hypertension. Aortic sensitivity to phenylephrine in ramipril-treated SHRSP was not significantly different from that in WKY rats, whereas untreated SHRSP were significantly more sensitive to phenylephrine than were WKY rats (unpublished). Furthermore, this vascular sensitivity did not directly correlate with terminal systolic blood pressure. SHRSP animals treated with hydral/HCTZ showed increased sensitivity to phenylephrine relative to WKY that was characteristic of an untreated SHRSP. Therefore, it was concluded that sensitivity to phenylephrine may reflect a primary defect associated with hypertension, and the altered sensitivity arises from an angiotensin II-mediated genetic program activated during development.

In the present study, contractile responsiveness to the Ca^{++} channel activators Bay K8644 and caffeine in ramipril-treated SHRSP were intermediate between those of untreated SHRSP and WKY and correlated with blood pressure. Thus, unlike phenylephrine, only a portion of the contractile responsiveness to Ca^{++} channel agonists was controlled by an angiotensin II-mediated developmental program. This may be due to the fact that Ca^{++} channel function is mediated and modulated by many different factors (e.g., G proteins, calmodulin-dependent kinases, Ca^{++} induced Ca^{++} release) and early angiotensin II inhibition may influence only some of these factors. Therefore, a decreased responsiveness to Ca^{++} channel agonists could explain the reduced blood pressure in ramipril-treated SHRSP. Other studies have suggested that the regulation of intracellular Ca^{++} may play a primary role in the augmented vascular reactivity characteristic of hypertension (Bohr and Webb, 1984; Kwan, 1985; Morgan and Suematsu, 1990; Sprenger, 1985). An alternate explanation, however, is that altered Ca^{++} handling and/or sensitivity was secondary to the elevated blood pressure. According to this explanation, the reduced blood pressure observed in the ramipril-treated animal could be attributed to reduced adrenergic sensitivity or to other factors mediated by the angiotensin II-sensitive program in development. The lower blood pressure of the ramipril-treated animal could, in turn, be responsible for the reduced response to the Ca^{++} channel agonists. Studies have shown that several vascular abnormalities characteristic of hypertension are associated with elevated blood pressure (Bohr *et al.*, 1991; Rapp, 1982).

Observations by Sada *et al.* (1989) were relevant to the present study and help clarify which of these two explanations was more likely to be true. They reported that long-term treatment (20 weeks) with ACE inhibitors (beginning at 23 weeks of age) in SHR suppressed the abnormal contractile responsiveness to Ca^{++} channel agonists. Furthermore, SHR treated with hydralazine for a similar period showed the enhanced contractile responsiveness to Ca^{++} channel agonists characteristic of untreated SHR, despite having blood pressures that were normotensive (133 ± 5 mm Hg). This strongly suggested that alterations in Ca^{++} handling were not secondary to changes in blood pressure. Thus, we propose that the attenuation of blood pressure observed in SHRSP treated with ramipril is caused by the prevention of abnormal contractile responsiveness to Ca^{++}, which in turn is caused by treatment with ACE inhibitors during development.

The mechanism by which ACE treatment during development affects long-term changes in responsiveness to Ca^{++} channel activators in vascular smooth muscle remains unclear. Several studies have reported a relationship between angiotensin II and voltage-sensitive Ca^{++} channels. Hausdorff and Catt (1988) have demonstrated that angiotensin II can activate dihydropyridine-sensitive voltage-sensitive Ca^{++} channels in the rat. Aguilera and Catt (1986) have shown that a close correlation (r = .92) exists between nitrendipine-binding sites and angiotensin II receptors in different zones of the adrenal in rat, dog and cow. Buisson *et al.* (1992) reported that angiotensin II can modulate T-type Ca^{++} current in certain nondifferentiated cell lines, and several investigators have demonstrated that angiotensin II can inhibit an anomalous inward rectifying potassium current in mouse, rat and bovine cells (Kanazirska *et al.*, 1992; Kurtz and Penner, 1990). Studies have shown that angiotensin II causes vasoconstriction by directly inhibiting potassium-induced Ca^{++} signal generation (Balla *et al.*, 1991; Toro *et al.*, 1990). It is possible that blocking of angiotensin II production during a critical period of development alters the responsiveness of vascular smooth muscle to Ca^{++} channel activators through any of the above mechanisms.

In summary, this study demonstrated that SHRSP show greater contractile responsiveness and force development to the Ca^{++} channel activators, Bay K8644 and caffeine; SHRSP treated with ramipril for a brief period during development show responsiveness and force development to Bay K8644 and caffeine in adulthood that is intermediate between untreated

SHRSP responses and WKY responses, whereas there is no change with hydral/HCTZ treatment; and intermediate blood pressures correspond to the intermediate responsiveness to Ca^{++} channel activators in ramipril-treated SHRSP. These data support the hypothesis that there is a correlation between blood pressure and responsiveness to Ca^{++} channel activators in hypertension. We conclude that a portion of abnormal Ca^{++} handling in hypertension is mediated by an angiotensin II-sensitive genetic program activated during development and that the corresponding attenuation of blood pressure is secondary to altered Ca^{++} regulation.

References

AGUILERA, G. AND CATT K. J.: Participation of voltage-dependent calcium channels in the regulation of adrenal glomerulosa function by angiotensin II and potassium. Endocrinology **118**: 112–118, 1986.

BALLA, T., HOLLO, Z., VARNAI, P. AND SPAT, A.: Angiotensin II inhibits K(+)-induced Ca^{2+} signal generation in rat adrenal glomerulosa cells. Biochem J **273**(Pt 2): 399–404, 1991.

BOHR, D. F., DOMINICZAK, A. F. AND WEBB, R. C.: Pathophysiology of the vasculature in hypertension. Hypertension **18**: 5 suppl III. 69–75, 1991.

BOHR, D. F. AND WEBB, R. C.: Vascular smooth muscle function and its changes in hypertension. Am. J. Med. **77**: 3–16, 1984.

BUISSON, B., BOTTARI, S. P., DE GASPARO, M., GALLO-PAYET, N. AND PAYET, M. D.: The angiotensin AT2 receptor modulates T-type calcium current in non-differentiated NG108–15 cells. FEBS Lett. **309**: 161–164, 1992.

FURSPAN, P. B. AND BOHR, D. F.: Cell membrane permeability in hypertension. Clin. Physiol. Biochem. **6**: 122–129, 1988.

FURSPAN, P. B., LAMB, F. S., ROSS, P. V., WEBB, R. C. AND BOHR, D. F.: Calcium and vascular smooth muscle membrane in hypertension. Prog. Clin. Biol. Res. **219**: 225–243, 1986.

GIUDICELLI, J. F., FRESLON, J. L., GLASSON, S. AND RICHER, C.: Captopril and hypertension development in the SHR. Clin. Exp. Hypertens. **2**: 1083–1096, 1980.

HARRAP, S. B.: A developmental genetic mechanism involving angiotensin in spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. suppl., **19**: 19–22, 1992.

HARRAP, S. B., VAN DER MERWE, W. M., GRIFFIN, S. A., MACPHERSON, F. AND LEVER, A. F.: Brief angiotensin converting enzyme inhibitor treatment in young spontaneously hypertensive rats reduces blood pressure long term. Hypertension **16**: 603–616, 1990.

HAUSDORFF, W. P. AND CATT, K. J.: Activation of dihydropyridine-sensitive calcium channels and biphasic cytosolic calcium responses by angiotensin II in rat adrenal glomerulosa cells. Endocrinology **123**: 2818–2826, 1988.

HERMSMEYER, K. AND ERNE, P.: Cellular calcium regulation in hypertension. Am. J. Hypertension **2**: 655–658, 1989b.

HERMSMEYER, K., HATTON, D. A., KARANJA, N. AND MCCARRON, D.: Effects of dietary calcium on nimodipine-sensitive calcium channel function in stroke prone spontaneously hypertensive rats. Stroke **21**: 12 suppl. IV98–101, 1990.

HERMSMEYER, K., STUREK, M., MARVIN, W., MASON, R. AND PUGA, A.: Vascular muscle calcium channel modulation in hypertension. J. Cardiovasc. Pharmacol. **14**: suppl. 6: S45–S48, 1989.

KANAZIRSKA, M. V., VASSILEV, P. M., QUINN, S. J., TILLOTSON, D. L. AND WILLIAMS, G. H.: Single K+ channels in adrenal zona glomerulosa cells: II. Inhibition by angiotensin II: Am. J. Physiol. **263**: (4 Pt 1) E760–765, 1992.

KANAMURA, Y., ITOH, T. AND KURIYAMA, H.: Agonist actions of Bay K8644, a dihydropyridine derivative, on the voltage-dependent calcium influx in smooth muscle cells of the rabbit mesenteric artery. J. Pharmacol. Exp. Ther. **231**: 717–723, 1984.

KARAKI, H., KUBOTA, H. AND URAKAWA, N.: Mobilization of stored calcium for phasic contraction induced by norepinephrine in rabbit aorta. Eur. J. Pharmacol. **56**: 237–245, 1979.

KURTZ, A. AND PENNER, R.: Effects of angiotensin II on intracellular calcium and electrical function of mouse renal juxtaglomerular cells. Kidney Int. **30**: suppl. S51–S54, 1990.

KWAN, C. Y.: Dysfunction of calcium handling by smooth muscle in hypertension. Can. J. Physiol. Pharmacol. **63**: 366–374, 1985.

LEE, R. M., BERECEK, K. H., TSOPORIS, J., MCKENZIE, R. AND TRIGGLE, C. R.: Prevention of hypertension and vascular changes by captopril treatment. Hypertension **17**: 141–150, 1991.

MORGAN, K. G.: Role of calcium ion in maintenance of vascular smooth muscle tone. (abstract) Am. J. Cardiol. **59**: 24A–28A, 1987.

MORGAN, K. G. AND SUEMATSU, E.: Effects of calcium on vascular smooth muscle tone: (abstract) Am. J. Hypertension **3**: (12 Pt 2) 291S–298S, 1990.

MORTON, J. J., BEATTIE, E. C. AND MACPHERSON, F.: Angiotensin II receptor antagonist losartan has persistent effects on blood pressure in the young spontaneously hypertensive rat: lack of relation to vascular structure. J. Vasc. Res. **29**: 264–269, 1992.

PERRY, P. A. AND WEBB, R. C.: Agonist-sensitive calcium stores in arteries from steroid hypertensive rats. Hypertension **17**: 603–611, 1991.

RAM, C. V.: Calcium antagonists in the treatment of hypertension. Am. J. Med. Sci. **290**: 118–133, 1985.

RAPP, J. P.: A genetic locus (Hyp-2) controlling vascular smooth muscle response in spontaneously hypertensive rats. Hypertension **4**: 459–467, 1982.

RINALDI, G. AND BOHR, D.: Plasma membrane and its abnormalities in hypertension. Am. J. Med. Sci. **295**: 389–395, 1988.

ROUSSEAU, E. AND MEISSNER, G.: Single cardiac sarcoplasmic reticulum Ca^{2+}-release channel: Activation by caffeine. Am. J. Physiol. **256**:(2 Pt 2) H328–H333, 1989.

SADA, T., KOIKE, H. AND MIYAMOTO, M.: Long-term inhibition of angiotensin converting enzyme suppresses calcium channel agonist-induced contraction of aorta in hypertensive rats. Hypertension **14**: 652–659, 1989.

SCHWARTZ, M. L., ROTMENSCH, H. H., VLASSES, P. H. AND FERGUSON, R. K.: Calcium blockers in smooth-muscle disorders. Current status. Arch. Intern. Med. **144**: 1425–1429, 1984.

SCRIABINE, A., KAZDA, S., GARTHOFF, B. AND NICKERSON, P. A.: Pharmacological basis for use of calcium channel antagonists in hypertension. Angiology **39**:(1 Pt 2) 94–99, 1988.

SPRENGER, K. B.: Alteration of cellular calcium metabolism as primary cause of hypertension. Clin. Physiol. Biochem. **3**: 208–220, 1985.

TORO, L., AMADOR, M. AND STEFANI, E.: ANG II inhibits calcium-activated nels from coronary smooth muscle in lipid bilayers. Am. J. Physiol. **258**:(3 Pt 2) H912–H915, 1990.

URTHALER, F.: Role of calcium channel blockers in clinical medicine. Am. J. Med. Sci. **292**: 217–230, 1986.

VANHOUTTE, P. M.: Calcium-entry blockers, vascular smooth muscle and systemic hypertension. Am. J. Cardiol. **55**: 17B–23B, 1985.

Send reprint requests to: Oren Traub, Department of Physiology, The University of Michigan, 7813 Medical Science Building II, Ann Arbor, MI 48109-0622.