Calcium Influx and Vascular Reactivity in Systemic Hypertension

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Numerous studies have focused on functional vascular changes that characterize the hypertensive state. Recent evidence that suggests that increased vascular reactivity in hypertension is due to changes in the delivery of activator Ca^{++} through channels in the cell membrane will be reviewed. The primary evidence supporting this hypothesis comes from studies that characterize the effects of Ca^{++}-free solution and calcium channel blockers on contractile properties of isolated vascular smooth muscle. In the present study, experiments were performed to investigate the role of Ca^{++} influx in vascular contractions produced by interventions that cause membrane depolarization. Isometric tension development in helical strips of carotid arteries from stroke-prone spontaneously hypertensive rats in response to elevated K^+ and tetraethylammonium chloride was greater than that in carotid arteries from Wistar-Kyoto normotensive rats. The rate of tension development to K^+-free solution in carotid arteries from stroke-prone spontaneously hypertensive rats was faster than in Wistar-Kyoto normotensive rat arteries. Contractile responses to all 3 depolarizing interventions were reduced in arterial strips incubated in Ca^{++}-free solution containing the chelator ethylene glycol bis-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid and in arterial strips treated with the Ca^{++} channel blocker verapamil. These results are consistent with the hypothesis that constrictor stimuli that produce membrane depolarization cause an opening of Ca^{++} channels in the plasma membrane that are sensitive to the organic channel blockers. Further, a change in Ca^{++} permeability or membrane depolarizing mechanisms contributes to increased contractile responsiveness in carotid arteries of stroke-prone spontaneously hypertensive rats.

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Vascular reactivity, defined as the magnitude of the response to a constrictor stimulus, is increased in humans with hypertension and in animal models of hypertension. The response may be measured as a contraction in an isolated vascular segment, as a change in resistance in a perfused vascular bed or as a pressor effect in the intact animal (if cardiac output is unchanged). Increased vascular reactivity in hypertension is due to a combination of 2 separate changes: a functional alteration in vascular smooth muscle, which results in a greater contraction in response to a given stimulus, and a structural modification of the vascular wall (increased wall to lumen ratio), which causes a greater decrease in vessel radius for a given shortening of vascular smooth muscle cells. Folkow[1] has reviewed the experimental evidence indicating that structural changes are an adaptive response to increased arterial blood pressure and that these structural changes contribute significantly to the increased vascular reactivity observed in hypertension.

Numerous studies have been performed that investigate functional alterations in vascular smooth muscle from hypertensive animals.[2] Because the contractile state of vascular smooth muscle varies in proportion to the concentration of Ca^{++} in the cytoplasm,[3] many investigators have tested the hypothesis that increased vascular reactivity in hypertension is due to increased delivery of activator Ca^{++} through channels in the cell membrane.[2–6] This aspect of excitation-contraction coupling has been characterized by measurement of

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contractile responses in isolated vascular preparations under 2 experimental conditions: in the presence and absence of extracellular Ca^{++}, and after treatment with drugs that block membrane channels for Ca^{++}.

Figures 1 and 2 show 2 types of contractile responses that are altered in hypertension; both are dependent on the level of Ca^{++} in the external bathing medium. Isolated arterial strips from 2 kidney, 1 clip (2K-1C) renal hypertensive rats and normotensive controls were incubated for 10 minutes in Ca^{++}-free solution containing the Ca^{++} chelator EGTA.[6] After the incubation period, arterial segments from 2K-1C, but not normotensive control rats, contracted in response to Ca^{++} (Fig. 1). The period in Ca^{++}-free solution (1 mM EGTA) appears to alter the vascular smooth muscle membrane in strips from hypertensive rats in a way that causes it to become more permeable to Ca^{++}. The magnitude of this "leak" appears to vary with the vessel studied, with the order of greatest to the least leak being aorta, mesenteric artery and tail artery. In addition to a Ca^{++} leak, it appears that specific membrane channels that open in response to receptor activation are changed in the hypertensive state. Tail arteries from stroke-prone spontaneously hypertensive rats (SHRs) contract in an oscillatory manner in response to norepinephrine whereas those from normotensive rats do not (Fig. 2).[3] The oscillatory contractile activity is caused by an abnormal variation in K^+ efflux during stimulation with norepinephrine. The altered K^+ efflux appears to be related to Ca^{++} entry because lowering the concentration of Ca^{++} in the external medium decreases the magnitude of oscillatory activity.[7,8]

Further evidence that supports the hypothesis that altered membrane flux of Ca^{++} contributes to increased vascular reactivity in hypertension is available from studies using calcium channel blockers.[7-12] Lederballe Petersen et al[10] have observed that nifedipine caused a more pronounced relaxation of SHR aortic strips contracted with norepinephrine than those from Wistar-Kyoto normotensive rats. Similarly, in humans, calcium channel blockers produce greater depressor effects in hypertensive subjects than in normotensive subjects.[12] In addition to blocking Ca^{++} influx through membrane channels, these compounds may also inhibit other actions of Ca^{++} at membrane sites. In a recent study in our laboratory,[13] we observed that elevated Ca^{++} causes relaxation of tail arteries contracted with methoxamine (Fig. 3). The magnitude of relaxation in response to elevated Ca^{++} is less in tail arteries from SHRs compared with those from Wistar-Kyoto normotensive rats. D-600 inhibited the relaxation to elevated Ca^{++}, suggesting that D-600 either blocks Ca^{++} sites that produce membrane stabilization or inhibits transmembrane movement of Ca^{++} that may act intracellularly to stimulate K^+ efflux and inhibit contraction.

The goal of the current study was to investigate the role of Ca^{++} influx in altered contractile responses to depolarizing stimuli in vascular smooth muscle from stroke-prone SHRs. Three depolarizing interventions were used: elevated K^+ concentration, K^+-free conditions and treatment with the K^+ channel blocker tetraethylammonium chloride. The role of Ca^{++} influx in contractile responses to membrane depolarization was determined by measuring the responses before and after incubation in Ca^{++}-free solution and in the absence and presence of the calcium channel blocker verapamil.

Methods

Animals: Stroke-prone SHRs and Wistar-Kyoto normotensive rats (8 of each strain) were obtained from a colony in the Department of Anatomy and Cell Biology at The University of Michigan. The colony was derived from stock supplied by the National Institutes of Health. The rats were 4 to 6 months old at the time of experimentation. Systolic blood pressures were measured in the conscious state by a tail cuff method (pneumatic transducer).

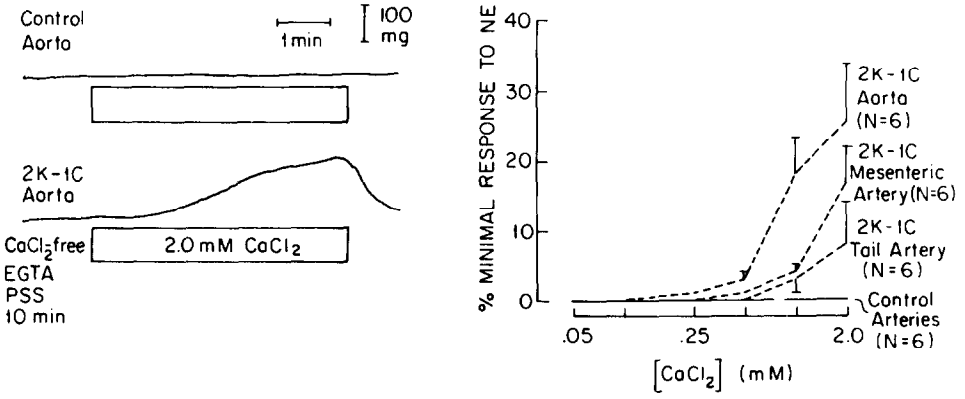

FIGURE 1. Vascular response after exposure to Ca^{++}-free physiologic salt solution (PSS) containing 1.0 mM EGTA. Arterial segments from 2 kidney, 1 clip (2K-1C) hypertensive, but not normotensive, rats contracted in response to Ca^{++} after treatment with Ca^{++}-free PSS, 1.0 mM EGTA (*left*) Contractile response (expressed as a percentage of the maximal response to norepinephrine) was greatest in aortic strips and least in tail arteries from hypertensive rats; mesenteric arteries were intermediate in contractile magnitude (*right*). Values are the mean ± standard error of the mean for 6 hypertensive and 6 normotensive rats. Reproduced with permission from Martinus Nijhoff.[6]

Experimental protocol: Rats were anesthetized with sodium pentobarbital (50 mg/kg) and both carotid arteries were excised and placed in a cold physiologic salt solution. Carotid arteries from stroke-prone SHRs and Wistar-Kyoto normotensive rats were cut into helical strips (0.8 mm × 10 mm). Vascular strips were mounted on stationary metal bases and suspended in a 50-ml tissue bath filled with physiologic salt solution maintained at 37°C. Arterial segments were attached to force transducers (Grass FT.03) for measurement of isometric force and recorded on Grass polygraphs. A constant passive tension of 5 mN was applied to the vascular strips and a 90-minute equilibration period preceded each experiment. The physiologic salt solution was aerated with a mixture of 95% O_2 and 5% CO_2 and its composition (mmol/liter) was as follows: NaCl (130), KCl (4.7), KH_2PO_4 (1.18), $MgSO_4 \cdot 7H_2O$ (1.17), $NaHCO_3$ (14.9), $CaCl_2$ (2.5), dextrose (5.5) and $CaNa_2EDTA$ (0.03). K^+-free solution was made by the omission of KCl and equimolar substitution of NaH_2PO_4 for KH_2PO_4 in the physiologic salt solution.

All experiments were conducted in the presence of phentolamine (Regitine mesylate, CIBA Pharmaceutical Co., 10^{-6} M) to eliminate vascular effects of norepinephrine that may be released from adrenergic nerve endings by depolarizing stimuli.[14] Elevations in K^+ concentration of the physiologic salt solution were made by addition of appropriate amounts from a concentrated solution of KCl (3 M).

Three vascular responses were examined in carotid arteries from stroke-prone SHRs and Wistar-Kyoto normotensive rats (6 of each): (1) dose-response curve to cumulative addition of KCl (8 to 125 mM) to the tissue bath, (2) time course of contraction in K^+-free physiologic salt solution (120 minutes exposure), and (3) cumulative dose-response curve to tetraethylammonium chloride (3×10^{-4}M to 10^{-1}M; Aldrich Chemical Co., Inc.). Vascular strips were exposed to each concentration of tetraethylammonium chloride for 20 minutes and responses were taken to be the peak contractile force at each concentration. Further experiments were performed in arteries from stroke-prone SHRs and Wistar-Kyoto normotensive rats to examine the effects of verapamil and Ca^{++}-free conditions on the just described vascular responses. Vascular strips were either incubated in Ca^{++}-free physiologic salt solution containing 1 mM EGTA or exposed to verapamil (10^{-6}M; Sigma Chemical Co.) for 5 minutes before the following stimuli: (1) 125 mM KCl, (2) 120 minutes exposure to K^+-free physiologic salt solution and (3) 10^{-1} M tetraethylammonium chloride. Peak contractile responses were analyzed.

To control for variations in strip size, isometric force was converted to tension. First, the area of each carotid artery strip was calculated as the weight of the tissue (mg) divided by the product of density (1.05) and length of the strip at 5 mN passive force (mm). Tension (mN/mm^2) was then calculated as isometric force (mN) divided by area (mm^2).

Statistical analysis: Data are reported as the mean ± standard error of the mean. For calculation of median effective dose values (concentration that caused a 50% maximal response) to KCl, contractile responses were expressed as a percentage of the maximal re-

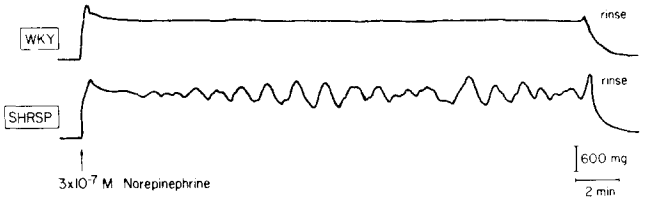

FIGURE 2. Oscillatory contractile responses to norepinephrine in tail arteries from stroke-prone spontaneously hypertensive rats (SHRSP). Norepinephrine (3×10^{-7}M) responses in isolated tail artery strips from SHRSP (*bottom*) are characterized by fluctuations in contractile activity, whereas contractile responses in arterial strips from Wistar-Kyoto (WKY) normotensive rats are maintained. Reproduced with permission from Am J Physiol.[7]

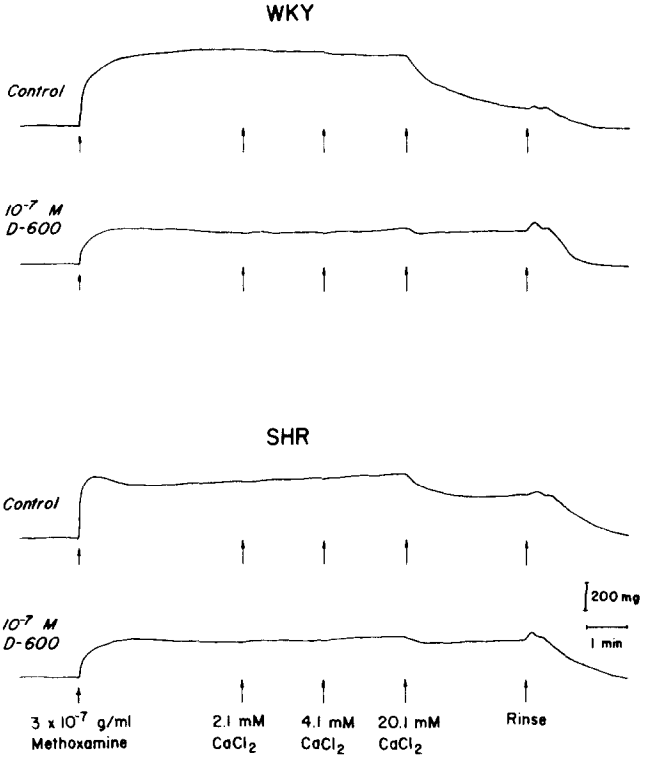

FIGURE 3. D-600 and the membrane stabilizing effect of Ca^{++}. Contractile responses to methoxamine (3×10^{-7} g/ml) were measured in tail artery strips from spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive rats in the absence and presence of 10^{-7}M D-600. After contractile responses had reached a plateau, the Ca^{++} concentration of the buffer was raised from 1.6 mM to 4.1 mM and then to 20.1 mM. Relaxation responses to 20.1 mM Ca^{++} were less in tail arteries from SHRs compared with that in tail arteries from WKY normotensive rats. This relaxation response is caused by a membrane stabilizing effect of Ca^{++} and is inhibited by D-600. The magnitude of D-600 inhibition of the Ca^{++}-induced relaxation is less in tail arteries of SHRs compared with WKY normotensive rats strips. Reproduced with permission from Alan R. Liss.[13]

sponse and a probit analysis was then performed. Threshold values for contractile responses to tetraethylammonium chloride are defined as the dose of the drug that produced a 5 mN/mm^2 response in the tissue, and these values were estimated after graphic representation of dose-response curves. Threshold values are used as an indicator of vascular sensitivity to tetraethylammonium chloride, because this agent did not cause maximal tension development. The rate of contraction in response to K^+-free solution is operationally defined as time to half of maximal response. Statistical comparisons between rat groups were performed by Student's *t* test. A p value of 0.05 was the criterion for statistical significance.

Results

Animals and tissue preparation: Systolic blood pressures of stroke-prone SHRs (196 ± 6 mm Hg, n = 8) were significantly higher than those of Wistar-Kyoto normotensive rats (118 ± 3 mm Hg, n = 8, $p < 0.05$). The calculated cross-sectional areas of carotid artery strips from stroke-prone SHRs (0.108 ± 0.005 mm^2, n = 16) did not differ from those of arterial strips from Wistar-Kyoto normotensive rats (0.100 ± 0.005 mm^2, n = 16).

Contractile responses to potassium chloride, potassium ion-free solution and tetraethylammonium chloride: Carotid artery strips from stroke-prone SHRs and Wistar-Kyoto normotensive rats developed tension in a concentration-dependent manner upon cumulative addition of KCl to the tissue bath (Fig. 4, left). The concentration of KCl that produced a half-maximal increase in tension (ED_{50}) was significantly lower for vascular strips from stroke-prone SHRs (20 mM KCl; − log ED_{50} = 1.29 ± 0.02, n = 6) when compared with that in strips from Wistar-Kyoto normotensive rats (34 mM KCl; - log ED_{50} = 1.53 ± 0.04, n = 6). The time to half of maximal response for contraction in K^+-free solution was significantly shorter in carotid arteries from stroke-prone SHRs (51 ± 2 minutes, n = 6) when compared with that in arteries from Wistar-Kyoto normotensive rats (70 ± 2 minutes, n = 6, Fig. 4, center). In contrast, maximal tension, developed after 125 mM KCl or a 120-minute exposure to K^+-free solution, was similar in both strains of rats.

Cumulative addition of tetraethylammonium chloride to the muscle bath caused increases in tension in vascular strips from stroke-prone SHRs (n = 6) and Wistar-Kyoto normotensive rats (n = 6) (Fig. 4, right). Stroke-prone SHRs (threshold value = 2.5 × 10^{-4}M; − log threshold value = 3.61 ± 0.18) were more sensitive to the effects of tetraethylammonium chloride than Wistar-Kyoto normotensive rats (threshold value = 9.8 × 10^{-3}M; − log threshold value = 2.01 ± 0.09, n = 6).

Contractile responses to 124 mM KCl, 120-minute exposure to K^+-free solution and 10^{-1}M tetraethylammonium chloride were virtually abolished in Ca^{++}-free solution containing 1 mM EGTA in carotid arteries from both hypertensive and normotensive rats (Fig. 5). Verapamil (10^{-6}M) also markedly attenuated (by 70% to 80%) the contractile responses to all of the stimuli mentioned (Fig. 6). Verapamil had a similar effect on hypertensive and normotensive rats.

Discussion

This study provides evidence that increased vascular reactivity to depolarizing stimuli in hypertension is due to an alteration in the delivery of activator Ca^{++} through cell membrane channels. Carotid artery strips from stroke-prone SHRs were more sensitive to the contractile effects of elevated K^+ and to the K^+ channel blocker tetraethylammonium chloride than strips from Wistar-Kyoto normotensive rats. The rate of tension development in carotid arteries from stroke-prone SHRs placed in K^+-free solution was faster than that in arterial strips from Wistar-Kyoto normotensive rats. Contractile responses of arterial strips to all 3 experimental interventions were inhibited by incubation in Ca^{++}-free solution and by the calcium channel blocker verapamil. Although previous studies have demonstrated an increased vascular reactivity to elevated K^+ concentration, K^+-free conditions and many other constrictor stimuli,[2] to our knowledge this is the

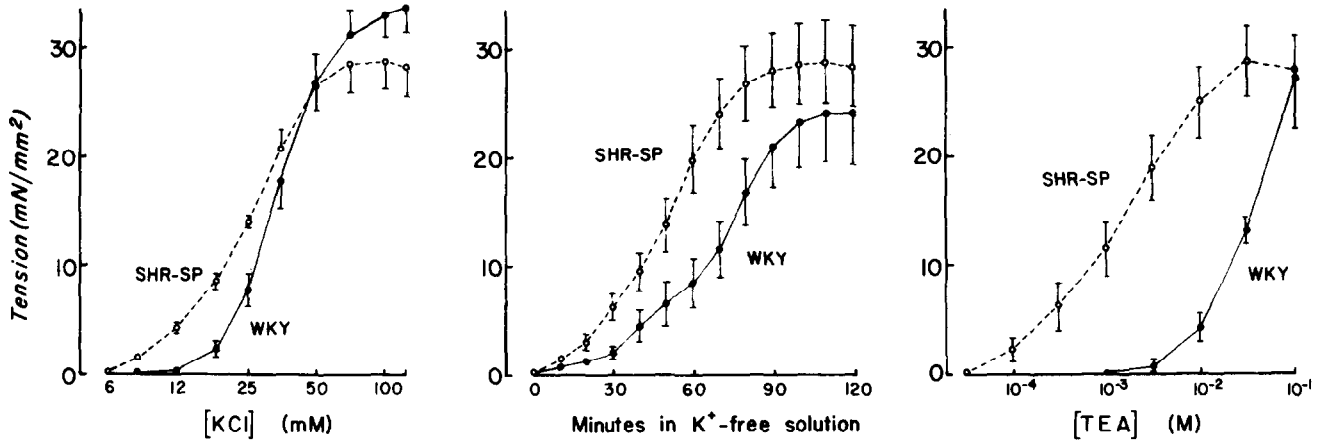

FIGURE 4. Vascular responses to elevated potassium (KCl), K^+-free solution and tetraethylammonium chloride (TEA). Carotid artery strips from stroke-prone spontaneously hypertensive rats (SHR-SP) were more sensitive to the contractile effects of KCl and tetraethylammonium chloride and contracted faster in response to K^+-free solution than arterial segments from Wistar Kyoto (WKY) normotensive rats. Values are mean ± standard error of the mean for 6 SHR-SP and 6 WKY normotensive rats.

first time that a greater sensitivity to tetraethylammonium chloride has been described.

Elevations in extracellular K^+ concentration produce membrane depolarization due to a reduction in the transmembrane gradient for the ion.[15] In contrast, membrane depolarization in response to K^+-free solution is caused by blockade of the electrogenic sodium pump[15] whereas tetraethylammonium chloride produces depolarization by inhibition of K^+ channels.[16] The observations of the current experiments indicate that the sensitivity to all 3 depolarizing stimuli is increased in carotid arteries from stroke-prone SHRs compared with Wistar-Kyoto normotensive rats. It seems likely that the primary membrane abnormality relates to a change in the relation between membrane potential and the opening of membrane channels for Ca^{++}. Altered regulation of potential-operated Ca^{++} channels in arteries from stroke-prone SHRs could contribute to the reduced threshold to tetraethylammonium chloride, the reduced ED_{50} values for K^+ and the faster rate of tension development to K^+-free incubation. Because maximal tension development to all 3 stimuli was not greater in arteries from stroke-prone SHRs compared with that in arteries from Wistar-Kyoto normotensive rats, it seems that the number of calcium channels that can be opened by a maximally effective depolarizing stimulus is not altered in arteries from stroke-prone SHRs. This speculation is strengthened by the observation that Ca^{++}-free conditions eliminated contractile responses to all 3 stimuli in arteries from both stroke-prone SHRs and Wistar-Kyoto normotensive rats.

There is a noteworthy characteristic of the dose-response curves for tension development to tetraethylammonium chloride in carotid arteries from stroke-prone SHRs. The threshold sensitivity to tetraethylammonium chloride was approximately 40 times lower in carotid arteries from stroke-prone SHRs compared with that in arteries from Wistar-Kyoto normotensive rats, whereas sensitivity to K^+ ion was increased by only a factor of 2 in carotid arteries from stroke-prone SHRs. Because tetraethylammonium chloride produces membrane depolarization by blocking K^+ channels, these observations suggest that the enhanced sensitivity to tetraethylammonium chloride may relate to altered membrane permeability to the monovalent ion.[16] Indeed, several studies have described increased membrane permeability to K^+ in vascular smooth muscle from hypertensive animals.[17] Alternatively, tetraethylammonium chloride may influence Ca^{++} conductance directly[16,18] and this effect may be altered in vascular smooth muscle from stroke-prone SHRs. The greater enhancement of the sensitivity to tetraethylammonium chloride relative to other depolarizing stimuli in hypertension is analogous to that of an enhanced receptor-mediated contraction induced by serotonin relative to other agents.[19] Vascular sensitivity to serotonin is enhanced to a greater degree in blood vessels from mineralocorticoid hypertensive rats than that to norepinephrine and angiotensin II.[19] The precise mechanisms responsible for relatively greater enhancement of constrictor responses to tetraethylammonium chloride and serotonin are unclear at the present time.

Current evidence supports the view that increased vascular reactivity in hypertension relates to an alter-

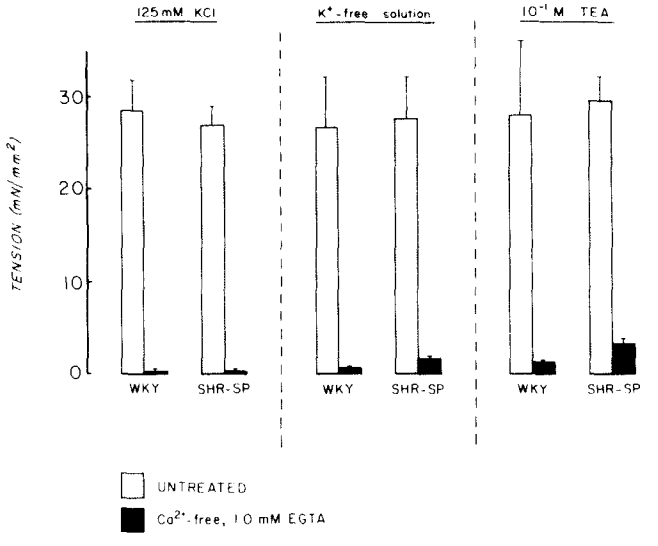

FIGURE 5. Ca^{++}-free solution and contractile responses to elevated K^+, K^+-free solution and tetraethylammonium chloride (TEA). Contractile responses in carotid artery strips from stroke-prone spontaneously hypertensive rats (SHR-SP) and Wistar Kyoto (WKY) normotensive rats to 125 mM KCl, K^+-free solution and 10^{-1}M tetraethylammonium chloride were inhibited when strips were placed in Ca^{++}-free solution containing 1.0 mM EGTA. Values are mean ± standard error of the mean for 4 SHR-SP and 4 WKY rats.

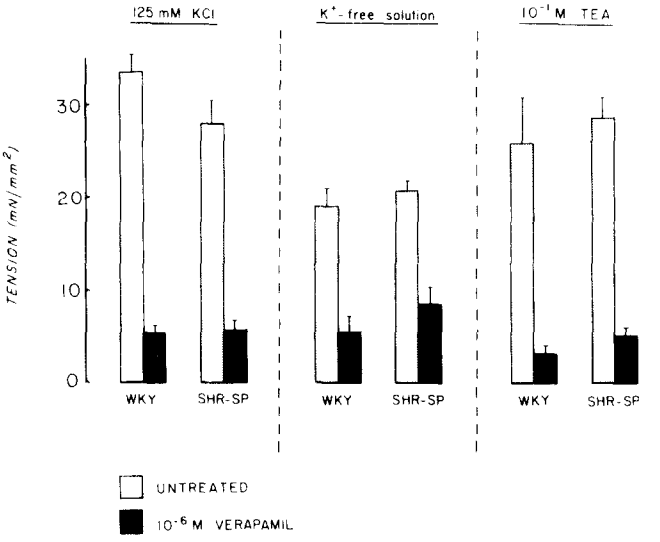

FIGURE 6. Verapamil and contractile responses to elevated K^+, K^+-free solution and tetraethylammonium chloride (TEA). Contractile responses to these 3 depolarizing stimuli in carotid artery strips from stroke-prone spontaneously hypertensive rats (SHR-SP) and Wistar Kyoto (WKY) normotensive rats were inhibited by 10^{-6}M verapamil. Values are mean ± standard error of the mean for 5 to 6 SHR-SP and 4 to 6 WKY rats.

ation in the transmembrane movement of Ca^{++}. This article characterized an increased sensitivity to depolarizing stimuli in arteries from rats with genetic hypertension. Because the contractile responses were blocked by verapamil and were absent in strips treated with Ca^{++}-free solution, it appears that contraction to the 3 depolarizing stimuli required external Ca^{++}. We conclude that in hypertension there is a generalized increase in sensitivity to depolarizing stimuli and a specific alteration in the membrane events that mediate the agonistic action of tetraethylammonium chloride.

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