Contractile Responses to Bay K 8644 in Rats with Coarctation-Induced Hypertension

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Abstract. **This study examines potential-operated calcium channel function in rats made hypertensive by aortic coarctation. The hypothesis that channel function is influenced by elevated arterial pressure was tested by comparing contractile responses to elevated K^+ and to the potential-operated calcium channel agonist, Bay K 8644, in aortic segments above (thoracic) and below (abdominal) the coarctation that are exposed to hypertensive and normotensive pressures, respectively. To control for vessel differences, the effects of Bay K 8644 were also examined in abdominal aortae from two-kidney, one-clip hypertensive rats. Sensitivity to K^+ (EC_{15}) was significantly greater in both thoracic and abdominal aortae from coarctation-hypertensive rats than in those from normotensive sham rats. In the thoracic aorta, maximal contractile response to Bay K 8644 (normalized to contraction produced by 100 m*M* K^+) was significantly greater in coarctation-hypertensive rats (124 ± 9%) than in sham rats (12 ± 6%). However, Bay K 8644 did not elicit contraction in abdominal aortae from either group. When $[K^+]_o$ was increased (19.2 m*M*), thoracic aortae from coarctation-hypertensive rats were more sensitive to Bay K 8644, but there were no differences in maximal responses among thoracic and abdominal aortae. Bay K 8644 evoked dose-dependent contraction in all abdominal aortic strips from two-kidney, one-clip hypertensive rats (maximum = 68 ± 11%). In summary, vascular responsiveness to Bay K 8644 is increased in the thoracic but not abdominal aorta from coarctation-hypertensive rats, whereas sensitivity to elevated K^+ is increased in both vessels. Enhanced K^+ sensitivity in the abdominal aorta may be related to general effects of the cation on membrane potential. However, augmented responsiveness to Bay K 8644 suggests a specific alteration in the function of potential-operated calcium channels that is dependent upon elevated blood pressure and is not due to differences in responsiveness between the thoracic and abdominal aortae.**

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Although it is well recognized that structural changes contribute to the increased reactivity that characterizes the hypertensive vasculature, the importance of changes in vascular smooth muscle function is also generally acknowledged. Several lines of evidence suggest that the permeability of potential-operated calcium channels is increased in hypertension. Arteries from hypertensive animals often develop spontaneous tone that is blocked by calcium channel antagonists, such as nicardipine (1, 2). A recent study by Smith and Jones (3) indicates that calcium channel blockade inhibits both basal tone and $^{45}Ca^{2+}$ influx in aortae from aldosterone-hypertensive rats. Blood vessels from hypertensive animals also exhibit increased sensitivity to depolarizing interventions, as demonstrated by either contractile responses (4, 5) or $^{45}Ca^{2+}$ influx (6). Furthermore, the dihydropyridine derivative, Bay K 8644, and structurally related calcium channel agonists induce contraction in isolated arteries from genetically hypertensive rats, but produce little, if any, contractile response in those from normotensive controls (7–10).

The effect of elevated arterial pressure on potential-operated calcium channel function in hypertension has not been clearly established. In deoxycorticosterone acetate-hypertensive rats, findings of increased con-

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tractile sensitivity to KCl in femoral arteries that have been protected from elevated pressure by ligation of the iliac artery suggest that alterations in channel function are not due to pressure-induced damage (11). Using isolated perfused kidneys from Dahl salt-sensitive and Dahl salt-resistant rats, Steele and Challoner-Hue (12) found that increased renovascular responsiveness to Bay K 8644 was genetically conferred in the salt-sensitive strain. However, they noted that responsiveness to the agonist was potentiated with the development of hypertension, implicating a role for elevated arterial pressure per se.

The present study examines potential-operated calcium channel function in arteries from rats with hypertension induced by coarctation of the abdominal aorta between the renal arteries. In this model, the role of increased wall stress in specific vascular abnormalities can be evaluated by comparing responses in vascular beds above and below the coarctation that have been exposed to hypertensive or normotensive levels of blood pressure, respectively (13). To test the hypothesis that potential-operated calcium channel function is influenced by elevated arterial pressure, contractile responses to KCl and Bay K 8644 were characterized in thoracic and abdominal aortic segments. Although the abdominal aorta lies below the coarctation and is protected from elevated pressure, it differs in several functional characteristics from the thoracic aorta. Therefore, in order to control for intrinsic differences between these vessels, responses to Bay K 8644 were also examined in abdominal aortae from rats with two-kidney, one-clip (2K1C) hypertension. In this model of renal hypertension, the abdominal aorta is exposed to elevated arterial pressure.

Methods

Animals. Adult, male Sprague-Dawley rats (Charles River, Portage, MI; 300–350 g) were made hypertensive by coarctation of the abdominal aorta. Animals were anesthetized with sodium pentobarbital (50 mg/kg, ip) and the abdominal aorta was ligated, using 5.0 silk suture, between the renal arteries. Control rats were sham operated with placement of a nonconstricting ligature around the abdominal aorta. Experiments were performed 2–3 weeks after surgery. At the time of experimentation, mean arterial pressure above the aortic coarctation was measured in anesthetized rats by catheterization of the common carotid artery. In some animals, mean arterial pressure was also measured below the coarctation by catheterization of the tail artery.

Two-kidney, one-clip renal hypertension was induced in 6-week-old, male Sprague-Dawley rats. The animals were anesthetized with ether and the left kidney was exposed through a flank incision. A silver clip (0.22-mm slit) was then placed on the left renal artery. Experiments were performed 6 months after surgery (550–650 g body wt at the time of experimentation). In these experiments, normotensive control rats did not undergo sham treatment. At the time of experimentation, systolic blood pressure was determined in the conscious state by indirect tail cuff method (pneumatic transducer). All animals used in this study were group housed in light-cycled (0600–1800 hr), temperature-controlled environments with free access to food and water.

Experimental Protocol. After blood pressure measurement, 2K1C rats were anesthetized with sodium pentobarbital (50 mg/kg, ip). All animals were then exsanguinated and the thoracic or abdominal aorta was removed and placed in cold physiologic salt solution (PSS). Vessels were cleaned of adherent fat and connective tissue and cut into helical strips (2 × 15 mm) under a dissecting microscope; the endothelium was left intact. Vascular strips were mounted vertically on stainless steel holders and placed in tissue baths filled with warmed (37°C), aerated (95% O_2 and 5% CO_2) PSS. The upper ends of the strips were connected to force transducers (FT.03; Grass, Quincy, MA) for measurement of isometric force. Recordings were made on a Grass polygraph. The composition of the PSS (in m*M*) was as follows: NaCl, 130; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$-$7H_2O$, 1.17; $CaCl_2$-$2H_2O$, 1.6; $NaHCO_3$, 14.9; dextrose, 5.5; and $CaNa_2$ EDTA, 0.03.

Vascular strips were equilibrated in PSS for 90 min at the start of each experiment, with passive forces of 1.5*g* and 1.0*g* placed on thoracic and abdominal aortic strips, respectively. Strips were then exposed to a 100-m*M* K^+ solution (made by equimolar substitution of KCl for NaCl in the PSS) until a stable force was reached. After return to baseline tension with a subsequent 60-min recovery period, concentration-response curves to Bay K 8644 were performed in a cumulative fashion. In some experiments, concentration-response curves to Bay K 8644 were performed in the presence of elevated K^+ (19.2 or 26.2 m*M*). Preliminary studies demonstrated that these concentrations of K^+ potentiated contractile responses to Bay K 8644 in the thoracic and abdominal aortae, respectively. The K^+ concentration was increased by the addition of an appropriate volume of a KCl stock solution (3 *M* KCl) 3–5 min before Bay K 8644. The subsequent contractile response to Bay K 8644 was measured from the level of contraction induced by the increase in K^+ concentration. Abdominal aortic strips were treated with phentolamine (10^{-6} *M*) prior to concentration-response curves in order to eliminate possible effects of norepinephrine released from nerve terminals.

Materials. Bay K 8644, a gift of Bayer (Wuppertal, Germany), was prepared as a 10^{-3} *M* stock solution in 95% ethanol. The concentration of ethanol in the tissue bath did not exceed 0.1%. Phentolamine (Regitine me-

sylate; Ben Venue Labs, Bedford, OH) was obtained from the University of Michigan Hospital Pharmacy. All other chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical Analysis. Data are expressed as means ± SE. Responses of each arterial strip were normalized to the force developed in 100 m*M* K^+. For calculation of concentration-response relationships, contraction was expressed as a percentage of the maximal response to the agonist. The concentrations of the agonist producing a threshold, or 15%, response (EC_{15}) and those producing a half-maximal response (EC_{50}) were then determined by graphical analysis. These values are expressed as negative logarithms. Statistical analyses were performed by Student's *t* test or by one-way analysis of variance using Fisher's protected least significant difference to test individual group comparisons. Dunnet's *t* test was used to determine differences from a single, designated comparison group. The criterion for statistical significance was a *P*-value of 0.05 or less.

Results

Mean arterial pressures (carotid artery) were significantly higher in rats with coarctation-induced hypertension (182 ± 3 mm Hg; $n = 17$) than in sham rats (126 ± 3 mm Hg; $n = 26$). Pressures recorded by cannulation of the tail artery did not differ between the two groups (coarctation: 98 ± 2 mm Hg, $n = 6$; sham: 113 ± 2 mm Hg, $n = 6$). Systolic blood pressures (tail cuff method) of 2K1C rats (209 ± 9 mm Hg, $n = 6$) were significantly greater than those of controls (113 ± 6 mm Hg, $n = 6$).

Concentration-Response Curves to K^+. Concentration-response curves to K^+ in coarctation-hypertensive and sham rats are shown in Figure 1. Sensitivity to K^+ was increased in thoracic (Fig. 1A) and abdominal (Fig. 1B) aortic strips from coarctation-hypertensive rats as compared with those from sham rats. In thoracic aortic strips, both the concentration of K^+ that produced a threshold contractile response (EC_{15}) and that producing a half-maximal response (EC_{50}) were significantly less in coarctation-hypertensive rats ($-\log EC_{15} = 2.01 \pm .04$, antilog = 9.8 m*M* K^+; $-\log EC_{50} = 1.84 \pm .05$, antilog = 14.5 m*M* K^+) than in sham rats ($-\log EC_{15} = 1.77 \pm .03$, antilog = 17 m*M* K^+; $-\log EC_{50} = 1.69 \pm .03$, antilog = 20.4 m*M* K^+). The threshold concentration of K^+ was also significantly lower in abdominal aortic strips from coarctation-hypertensive rats ($-\log EC_{15} = 1.72 \pm .05$; antilog = 19.1 m*M* K^+) as compared with those from sham rats ($-\log EC_{15} = 1.62 \pm .02$; antilog = 24 m*M* K^+). However, in these vessels, the half-maximal concentration of K^+ did not differ between the two groups (coarctation: $-\log EC_{50} = 1.55 \pm .05$, antilog = 28.2 m*M* K^+; sham: $-\log EC_{50} = 1.49 \pm .02$, antilog = 32.4 m*M* K^+). Comparison of EC_{15} and EC_{50} values for K^+ between thoracic and abdominal aortic segments indicated that thoracic aortic strips were more sensitive to K^+ than abdominal aortic strips in both coarctation-hypertensive and sham rats. In thoracic aortic strips, maximal force development to K^+ did not differ between coarctation hypertensive rats (704 ± 72 mg) and sham rats (631 ± 47 mg). Similarly, maximal force developed to K^+ was not significantly different in abdominal aortic strips from coarctation-hypertensive rats (502 ± 165 mg) than in those from sham rats (530 ± 67 mg).

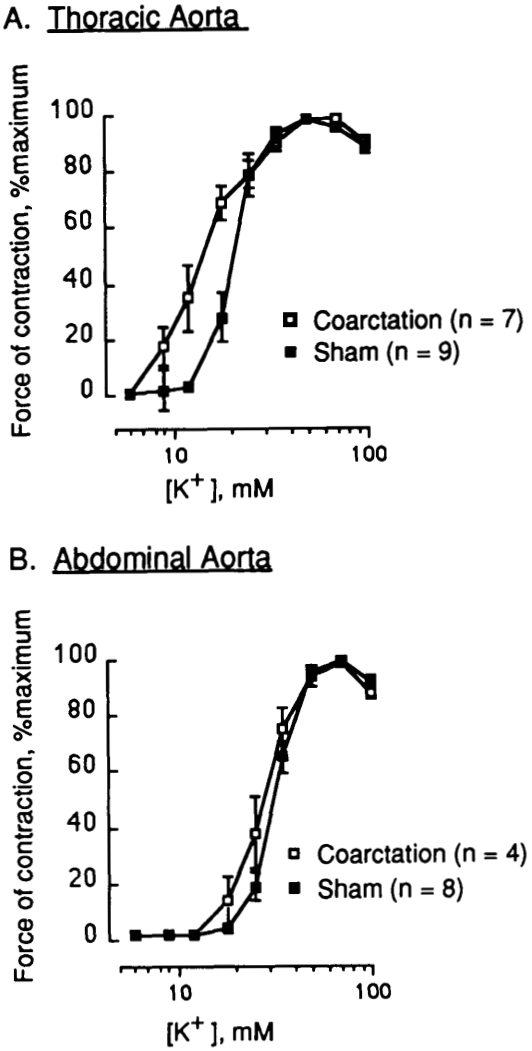

Figure 1. Concentration-response curves to K^+ in (A) thoracic and (B) abdominal aortic strips from coarctation-hypertensive (open symbols) and sham (closed symbols) rats. Points are mean values ± SE. See text for EC_{15} and EC_{50} values.

Effects of Bay K 8644 in Aortae from Coarctation-Hypertensive Rats. Concentration-response curves to Bay K 8644 are shown in Figure 2. Corresponding effective dose concentrations and maximal contractile responses are summarized in Table I. Cumulative addition of Bay K 8644 (10^{-9} to 3×10^{-7} *M*) elicited tonic force development in all thoracic aortic strips from coarctation-hypertensive rats, but produced

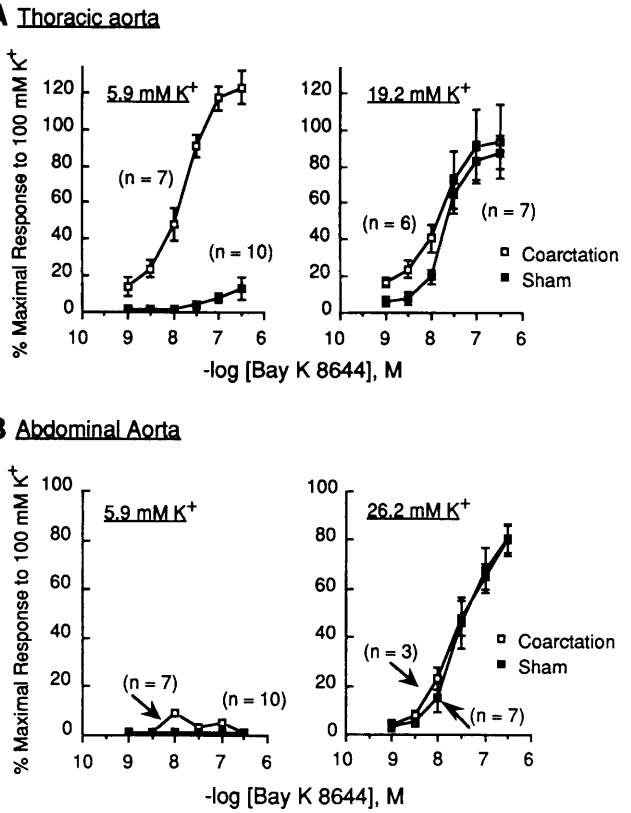

Figure 2. Concentration-response curves to Bay K 8644 in (A) thoracic and abdominal (B) aortic strips from coarctation-hypertensive (open symbols) and sham (closed symbols) rats. Left panels illustrate the effect of Bay K 8644 at a physiologic K^+ concentration (5.9 m*M*). Right panels show the effect of increased K^+ concentrations on concentration-response curves to Bay K 8644. Points are mean values ± SE. See Table I for corresponding EC_{15} and EC_{50} values.

tonic contractions in only six of 10 thoracic aortic strips from sham rats (Fig. 2A, left panel). With the exception of two vascular strips from coarctation-hypertensive rats, Bay K 8644 did not evoke any contractile activity in abdominal aortic strips from either coarctation-hypertensive or sham rats (Fig. 2B, left panel). Because Bay K 8644 alone did not elicit consistent contraction in thoracic aortae from sham rats or in abdominal aortae, effective dose concentrations were calculated only in those thoracic aortic strips in which Bay K 8644 evoked a maximal contractile response equivalent to at least 15% of the response to 100 m*M* K^+.

Among thoracic aortic strips that exhibited a tonic response to Bay K 8644, those from coarctation-hypertensive rats were more sensitive to Bay K 8644 than those from sham rats, as assessed by threshold concentrations of the agonist (Table I). However, the concentration of Bay K 8644 producing a half-maximal response did not differ significantly between the two groups. The maximal contractile response to Bay K 8644 in thoracic aortic strips from coarctation-hypertensive rats exceeded the response to 100 m*M* K^+ and was significantly greater than the maximal response to Bay K 8644 in thoracic aortic strips from sham rats or in abdominal aortic strips from either coarctation-hypertensive or sham rats (Table I; Fig. 2, left panels). There were no significant differences in the maximal response to Bay K 8644 among abdominal aortic strips from coarctation-hypertensive rats and thoracic or abdominal aortic strips from sham rats.

Elevation of the bath K^+ concentration to 19.2 m*M* increased the sensitivity and the maximal response to Bay K 8644 in thoracic aortic strips from sham rats, but did not significantly shift the concentration-response curve in those from coarctation-hypertensive rats (Table I; Fig. 2A). The contractile response produced by 19.2 m*M* K^+ alone was 34 ± 10% (percentage of maximal response to 100 m*M* K^+) in coarctation-hypertensive rats and 16 ± 5% in sham rats. In the presence of elevated K^+, the threshold concentration of Bay K 8644 was significantly less in thoracic aortic strips from coarctation-hypertensive rats than in those from sham rats; neither EC_{50} values nor maximal contractile responses were significantly different. With elevation of K^+ concentration to 26.2 m*M* in abdominal aortic strips, the sensitivity and the maximal response to Bay K 8644 increased in both coarctation-hypertensive and sham rats and did not differ between the two groups (Table I; Fig. 2B). The magnitude of contraction elicited by 26.2 m*M* K^+ alone was 9 ± 6% in coarctation-hypertensive rats and 12 ± 6% in sham rats. Although thoracic aortic strips from coarctation-hypertensive rats were more sensitive to Bay K 8644 than abdominal aortic strips from either coarctation-hypertensive or sham rats, the maximal contractile response to the agonist did not differ among the groups when the K^+ concentration was increased. There were no differences in the sensitivity or maximal response to Bay K 8644 among thoracic aortic strips from sham rats and abdominal aortic strips from either group of rats with elevation of K^+. In the presence of elevated K^+, the sensitivity and maximal response to the agonist in thoracic aortic strips from sham rats and in abdominal aortic strips from either coarctation-hypertensive or sham rats did not differ from those values observed with Bay K 8644 alone in thoracic aortic strips from coarctation-hypertensive rats (Table I).

Effects of Bay K 8644 in Abdominal Aortae from 2K1C Hypertensive Rats. Figure 3 illustrates concentration-response curves to Bay K 8644 in abdominal aortic strips from 2K1C hypertensive rats and normotensive control rats. Bay K 8644 (3×10^{-10}-3×10^{-7} *M*) produced dose-dependent contractile responses in strips from 2K1C rats, but did not evoke contraction in those from control rats (Fig. 3, left panel). In abdominal aortae from 2K1C rats, where arterial pressure is elevated, the maximal contractile response to Bay K 8644 was significantly greater than that in abdominal aortae from control, coarctation-hypertensive, or sham

Table I. EC_{15} and EC_{50} Values for Bay K 8644 and Maximal Contractile Responses to Bay K 8644[a]

	−Log EC_{15} (*M*)	−Log EC_{50} (*M*)	% Maximal response to 100 m*M* K^{+b}
Thoracic aorta—5.9 m*M* K^+			
Coarctation (n = 7)	8.79 ± 0.16 (2.00×10^{-9})	7.82 ± 0.13 (1.51×10^{-8})	124 ± 9
Sham (n = 10)	7.68 ± 0.26 (2.09×10^{-8})[c,d]	7.32 ± 0.23 (4.78×10^{-8})[c]	12 ± 6[d]
Thoracic aorta—19.2 m*M* K^+			
Coarctation (n = 6)	8.81 ± 0.12 (1.55×10^{-9})	8.07 ± 0.14 (8.51×10^{-9})	94 ± 2
Sham (n = 7)	8.31 ± 0.17 (4.90×10^{-9})	7.68 ± 0.09 (2.09×10^{-8})	89 ± 9
Abdominal aorta—5.9 m*M* K^+			
Coarctation (n = 7)	—	—	11 ± 9[d,e]
Sham (n = 10)	—	—	0[d,e]
2K1C (n = 6)	8.54 ± 0.24 (2.88×10^{-9})	7.79 ± 0.20 (1.62×10^{-8})	68 ± 11[d,e]
Control (n = 6)	—	—	0[e]
Abdominal aorta—26.2 m*M* K^+			
Coarctation (n = 3)	7.98 ± 0.34 (1.05×10^{-8})	7.47 ± 0.15 (3.39×10^{-8})	81 ± 6
Sham (n = 7)	8.15 ± 0.17 (7.10×10^{-9})	7.53 ± 0.11 (2.95×10^{-8})	81 ± 6
Abdominal aorta—19.2 m*M* K^+			
2K1C (n = 4)	9.42 ± 0.68 (8.81×10^{-10})[e]	8.30 ± 0.31 (8.51×10^{-9})[e]	95 ± 3
Control (n = 6)	8.50 ± 0.07 (3.16×10^{-9})	7.92 ± 0.04 (1.20×10^{-8})	31 ± 9[e]

[a] Data are expressed as mean ± SE, with molar concentrations for corresponding mean EC_{15} and EC_{50} values (antilog) denoted in parentheses.
[b] Responses to Bay K 8644 are expressed as a percentage of the maximal contractile response to 100 m*M* K^+.
[c] Based on aortic strips responding to Bay K 8644 with a maximal response of at least 15% (n = 4).
[d] In comparisons performed among thoracic and abdominal aortic strips from coarctation-hypertensive and sham rats by one-way analysis of variance, there was a significant difference from value for coactation thoracic aorta by Dunnet's t test.
[e] In comparisons performed among thoracic and abdominal aortic strips from all rat groups by one-way analysis of variance, there was a significant difference from value for two-kidney, one-clip abdominal aorta by Dunnet's t test.

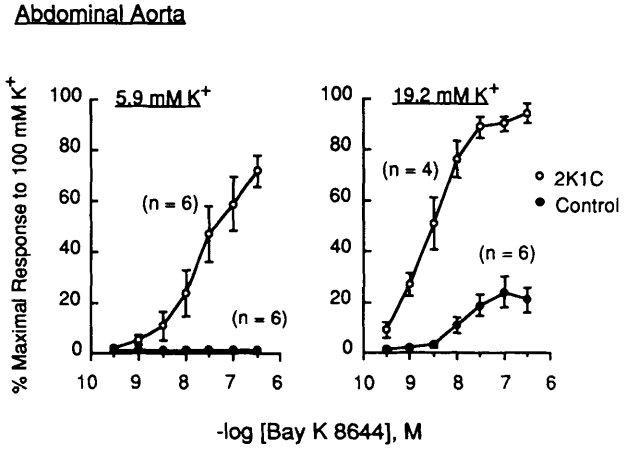

Figure 3. Concentration-response curves to Bay K 8644 in abdominal aortic strips from 2K1C (open symbols) and control rats (closed symbols). The left panel illustrates the effect of Bay K 8644 at a physiologic K^+ concentration (5.9 m*M*). The right panel shows the effect of an increased K^+ concentration on concentration-response curves to Bay K 8644. Points are mean values ± SE. See Table I for corresponding EC_{15} and EC_{50} values.

rats, vessels that are exposed to normotensive arterial pressures (Table I; compare left panels of Figs. 2B and 3).

When the K^+ concentration was raised, the sensitivity and maximal response to Bay K 8644 were increased (Table I; compare right panels of Figs. 2B and 3), and the agonist induced a dose-dependent contraction in all abdominal aortic strips tested. In the presence of 19.2 m*M* K^+, sensitivity to Bay K 8644, as assessed by threshold concentration, and the maximal response to the agonist were significantly greater in abdominal aortae from 2K1C rats than in those from control rats; EC_{50} values did not differ. The contraction produced by 19.2 m*M* K^+ alone was 16 ± 6% in 2K1C rats and 2 ± 1% in control rats. With elevated K^+, sensitivity to Bay K 8644 was also greater in abdominal aortic strips from 2K1C rats (19.2 m*M* K^+) as compared with those from coarctation-hypertensive or sham rats (26.2 m*M* K^+), as assessed by both EC_{15} and EC_{50} values, although maximal responses to the agonist did not differ among these groups. While sensitivity to Bay K 8644 was not different among coarctation-hypertensive, sham, and control rats when the K^+ concentration was increased, the maximal response to the agonist was significantly greater in abdominal aortic strips from coarctation-hypertensive and sham rats (26.2 m*M*) than in those from control rats (19.2 m*M*). In abdominal aortae from coarctation-hypertensive, sham, or control rats, sensitivity to Bay K 8644 in the presence of elevated potassium did not differ from sensitivity to Bay K 8644 alone in 2K1C aortae (Table I). In coarctation-hypertensive or sham rats, the maximal response to the agonist in the presence of 26.2 m*M* K^+ was not significantly different from that elicited by Bay K 8644 alone in 2K1C rats. However, in the presence of 19.2 m*M* K^+, the maximal contractile response produced by Bay K 8644 in abdominal aortic strips from control rats was significantly less than that evoked by Bay K 8644 alone

in those from 2K1C rats. The force of contraction to 100 mM K^+ was significantly greater in abdominal aortic strips from control rats (1006 ± 58 mg; $n = 6$) than in those from 2K1C (634 ± 76 mg; $n = 6$), coarctation-hypertensive (522 ± 79 mg; $n = 6$), or sham (622 ± 78 mg; $n = 11$) rats; there were no differences in the force of contraction among the remaining groups.

Discussion

Enhanced vascular sensitivity to depolarizing concentrations of KCl has been documented in both genetic and experimental models of hypertension (4, 5). However, augmented responsiveness to Bay K 8644 and other potential-operated calcium channel agonists has been reported only in arteries from genetically hypertensive rats (7–10). The present study demonstrates that contractile responsiveness to Bay K 8644 is markedly increased in thoracic aortae from coarctation-hypertensive rats as compared with those from normotensive sham rats. In the abdominal aorta, the agonist does not elicit consistent contraction in either coarctation-hypertensive or sham rats. These findings support the hypothesis that augmented vascular responsiveness to Bay K 8644 in hypertension represents a pressure-sensitive defect, since the abdominal aorta is protected from elevated blood pressure in coarctation-induced hypertension. However, because the effect of Bay K 8644 has been shown to vary in different vessels (7), responses to the agonist were also characterized in abdominal aortae from 2K1C hypertensive rats. In the 2K1C model of renal hypertension, which is comparable to that produced by coarctation of the abdominal aorta, the abdominal aorta is exposed to elevations in arterial pressure. Contractile responsiveness to Bay K 8644 is increased in abdominal aortae from 2K1C rats as compared with those from coarctation-hypertensive rats or control rats, indicating that the abdominal aorta does exhibit altered responsiveness to the agonist when it has been exposed to elevated arterial pressure. Thus, it can be concluded that enhanced responsiveness to Bay K 8644 in thoracic but not abdominal aortae from rats with coarctation-induced hypertension is related to differences in the arterial pressure between the two vessels rather than to intrinsic differences in agonist responsiveness.

It has been postulated that augmented vascular responsiveness to calcium channel agonists in hypertension is due to an alteration in the function of potential-operated calcium channels (7, 8). This conclusion is based on observations showing that the effects of this class of calcium channel agonists are dependent upon extracellular calcium and can be inhibited by calcium channel antagonists (14). In addition, Bay K 8644 has been found to augment whole cell calcium currents in isolated vascular smooth muscle cells (15). More recently, it has been reported that enhanced contractile responses to the agonist, CGP-28392, in thoracic aortae from SHR are coupled to increases in $[Ca^{2+}]_i$, offering further support to the postulated defect in calcium channel function (16).

A number of studies have implicated effects of elevated arterial pressure per se in the development of hypertensive vascular changes (17, 18). Previous work from our laboratory demonstrated that sensitivity to the protein kinase C activator, 12-*O*-tetradecanoylphorbol 13-acetate, was increased in thoracic but not abdominal aortae from coarctation-hypertensive rats (19). However, Bell and Overbeck (20) found that minimal vascular resistance was elevated in the hindquarters of rats with coarctation-induced hypertension, indicating that changes may also occur in normotensive vascular beds. In the present study, sensitivity to K^+, assessed by threshold concentration, was increased in both thoracic and abdominal aortae. Based on K^+ sensitivity, altered potential-operated calcium channel function in coarctation-induced hypertension appears to be independent, at least in part, from the effects of elevated blood pressure. However, in contrast to Bay K 8644, which acts specifically on calcium channels, elevation of extracellular K^+ has a general effect on the plasma membrane. Reductions in the transmembrane K^+ gradient and subsequent depolarization affect all K^+ and voltage-dependent processes. Indeed, our findings suggest that changes in K^+ sensitivity alone do not provide a specific index of alterations in potential-operated calcium channel function.

As others have noted (7, 8, 14), we found that sensitivity and maximal responses to Bay K 8644 could be increased in the presence of an elevated K^+ concentration, which acts to partially depolarize the plasma membrane. While an elevation in K^+ concentration potentiated responses to Bay K 8644 in thoracic aortae from sham rats and in abdominal aortae from all groups of rats, it did not shift the concentration response curve to the agonist in thoracic aortae from coarctation-hypertensive rats. This apparent lack of effect may be due to the K^+ concentration used and the magnitude of contraction elicited by increased K^+ alone. However, there were no differences in the sensitivity and maximal response to Bay K 8644 between thoracic aortae from coarctation-hypertensive and those from sham rats in the presence of 19.2 mM K^+. With elevation of potassium to 19.2 mM, both sensitivity and maximal responsiveness to Bay K 8644 were significantly greater in abdominal aortic strips from 2K1C hypertensive rats than in those from normotensive control rats. When the K^+ concentration was increased to 26.2 mM, the maximal response to Bay K 8644 in abdominal aortae from either coarctation-hypertensive or sham rats did not differ from that observed in abdominal aortae from 2K1C rats in the presence of 19.2 mM K^+, although 2K1C aortae were still more sensitive to the agonist.

Because responses to Bay K 8644 in arteries from normotensive animals can be potentiated with elevations in K^+ concentration, it has been suggested that a decrease in resting membrane potential could account for enhanced responsiveness to Bay K 8644 in hypertension (7, 9). Such a change could also explain increased sensitivity to KCl and other depolarizing stimuli. While a few studies have reported that vascular smooth muscle from hypertensive animals is depolarized with respect to that from normotensive controls (21, 22), a number of others have found that resting membrane potential is not altered in hypertension either at physiologic or increased concentrations of K^+ (23–26). Moreover, it has been shown that renovascular resistance changes produced by Bay K 8644 are increased in isolated perfused kidneys from Dahl salt-sensitive rats, but that sensitivity to KCl does not differ from that observed in normotensive Dahl salt-resistant rats (12). This finding argues against the interpretation that enhanced responsiveness to Bay K 8644 in hypertension can be attributed solely to changes in membrane potential.

Although potential-operated calcium channels are gated by changes in membrane potential, evidence indicates that they may also be modulated by a number of other factors, including receptor-mediated phosphorylation by cyclic AMP or direct coupling with G proteins (27, 28). It has been postulated that, at resting membrane potential, more calcium channels are in a configuration to bind Bay K 8644 in arteries from hypertensive animals than in those from controls, but that this difference can be eliminated when the membrane is sufficiently depolarized (8). A recent study by Godfraind *et al.* (29) examined specific binding of the dihydropyridine calcium channel antagonist, ^{3}H-(+) PN 200-110, in intact aortae from SHR and WKY rats over a range of KCl concentrations. It was found that at 6 m*M* KCl, the apparent K_d was significantly lower in arteries from SHR than in those from WKY rats, but at 100 m*M* KCl, the K_d was reduced and did not differ between the two strains of rats. At physiologic KCl concentrations, it was calculated that 30% of calcium channels in aortae from SHR were in a high affinity configuration, but that only 5% exhibited this configuration in aortae from WKY rats. These findings suggest that the greater sensitivity to dihydropyridines observed in SHR under physiologic conditions is related to changes in the conformational state of potential-operated calcium channels.

This study demonstrates that reactivity to Bay K 8644 is increased in the thoracic, but not the abdominal, aorta from coarctation-hypertensive rats, whereas sensitivity to elevated K^+ is increased in both vessels. Enhanced sensitivity to K^+ in the abdominal aorta may be related to general effects of the cation on membrane potential. However, augmented responsiveness to Bay K 8644 suggests the presence of a specific alteration in the function of potential-operated calcium channels. This change is due to an effect that is dependent upon blood pressure since it occurred in the thoracic aorta above the coarctation but not in the abdominal aorta below the coarctation. The finding that responsiveness to Bay K 8644 is increased in abdominal aortae from 2K1C hypertensive rats provides further support for the interpretation that altered potential-operated calcium channel function in hypertension is related to the effects of elevated arterial pressure.

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