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Effects of Bay K 8644 and nifedipine on femoral arteries of spontaneously hypertensive rats

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1 Vasoconstrictor effects of Bay K 8644 (an agonist known to increase Ca^{2+} influx through the voltage-dependent Ca^{2+} channels) on femoral arteries of 6 week old spontaneously hypertensive rats (SHR) were investigated, and data compared with findings in age-matched normotensive Wistar-Kyoto rats (WKY).

2 The addition of Bay K 8644 (1×10^{-10}–3×10^{-7} M) elicited a dose-dependent contraction in SHR femoral artery in the absence of any contractile agent. Maximum contraction induced by this agonist was the same as the maximum induced by either K^+-depolarization or α-adrenoceptor stimulation.

3 Bay K 8644 was less effective in eliciting a contraction in the WKY femoral artery.

4 Increased sensitivity to K^+ was also observed in the SHR femoral artery. In contrast, contractions in response to α-adrenoceptor stimulation were the same in the SHR as those in the WKY.

5 The addition of nifedipine, a Ca^{2+} channel antagonist, to an unstimulated preparation produced a dose-dependent relaxation in femoral arteries from SHR, but not from WKY. When the arteries were contracted with 60 mM K^+, nifedipine produced similar relaxations in the SHR as those in the WKY, suggesting that the Ca^{2+} channels in the SHR femoral arteries are more activated than those in the WKY femoral arteries.

6 Contractile responses of SHR femoral arteries to Bay K 8644 were antagonized competitively by nifedipine.

7 Contractile responses to Ca^{2+} determined in K^+-depolarized strips were also antagonized competitively by nifedipine. However, Schild plot analysis clearly demonstrated a different pA_2 value for nifedipine, suggesting that there may be a difference in the state of voltage-dependent Ca^{2+} channels in SHR femoral artery between the stimulation with Bay K 8644 and K^+-depolarization.

Introduction

The elevated arterial pressures of spontaneously hypertensive rats (SHR) and patients with essential hypertension are caused by an increased total peripheral resistance. Abnormal responses to a variety of vasoconstrictor and vasodilator agents have been demonstrated in arterial smooth muscle isolated from hypertensive animals, including SHR when compared to normotensive animals (Webb & Bohr, 1981; Webb *et al.*, 1981; Daniel & Kwan, 1981; Winquist *et al.*, 1982, Mulvany, 1983). Such abnormalities have been related to the initiation and maintenance of the increased total peripheral resistance associated with hypertension in the SHR. The cellular mechanisms responsible for the abnormal vascular smooth muscle function in SHR are not clear, but one of the most consistent findings in the vascular smooth muscle of SHR is increased Ca^{2+} handling properties (Webb & Bohr, 1981). Holloway & Bohr (1973) have demonstrated that femoral arterial strips isolated from animals with various types of hypertension including SHR are more sensitive to exogenously applied KCl (K^+) than those from normotensive animals. Because the response of vascular smooth muscle to K^+ is well known to depend on Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Bolton, 1979), the increase in K^+ sensitivity suggests an increased permeability of plasma membrane to Ca^{2+}.

Dihydropyridine derivatives, such as nifedipine, are specific Ca^{2+} channel antagonists, i.e. they block the influx of Ca^{2+} through the voltage-dependent Ca^{2+} channels in vascular smooth muscle (Fleckenstein,

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1977; 1983), and thus are used therapeutically as potent vasodilators in cardiovascular disorders (Krebs, 1984). Recently, a novel dihydropyridine derivative, Bay K 8644 (methyl-1,4-dihydro- 2,6-dimethy-3-nitro-4- (2-trifluoromethylphenyl)-pyridine-5-carboxylate), has been shown to have effects opposite to those of nifedipine (Schramm *et al.*, 1983a,b). Bay K 8644 has vasoconstrictor and positive inotropic effects. Recent studies have confirmed agonist actions of Bay K 8644 on voltage-dependent Ca^{2+} channels in various vascular smooth muscle preparations (Yamamoto *et al.*, 1984; Su *et al.*, 1984; Kanmura *et al.*, 1984; Uski & Andersson, 1985; Mikkelsen *et al.*, 1985; Gopalakrishnan *et al.*, 1985; Salaices *et al.*, 1985). Therefore, in the present study, we have examined the agonist actions of Bay K 8644 on femoral arterial strips isolated from 6 week old SHR, that is an early stage of hypertension, and age-matched normotensive Wistar-Kyoto rats (WKY).

Methods

Preparation of femoral arterial strips for tension recordings

Male SHR, 6 weeks of age, and age-matched male WKY were used. Systolic blood pressures, measured in conscious rats by the tail-cuff method (Okamoto & Aoki, 1963), were 134 ± 8 mmHg (SHR, $n = 20$) and 114 ± 3 mmHg (WKY, $n = 20$, significantly different from SHR), respectively.

The rats were stunned and exsanguinated. Femoral arteries (0.5–0.7 mm outside diameter) were quickly dissected. After removal of adhering fat and connective tissue, the arteries were helically cut into strips of 0.8 mm in width and 7 mm in length, according to the method of Furchgott & Bhadrakom (1953). The strips were mounted vertically in all-glass 20 ml muscle chambers at 37 ± 0.5°C for isometric recording of tension. The bathing solution was modified Krebs bicarbonate solution of the following composition (mM): NaCl 115.0, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and dextrose 10.0. The solutions were continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The upper end of the strip was connected to the lever of a force-displacement transducer (TB-612T, Nihon Kohden Kogyo Co., Tokyo, Japan) and the strips were stretched passively by imposing a resting tension of 0.4 g. This tension was maintained throughout the experiments. This degree of passive stretch of strips from SHR and WKY is nearly optimal for active tension development. Length-passive tension studies failed to demonstrate differences in passive stiffness between strips from SHR and WKY. After application of resting tension, strips were equilibrated for 90 min in oxygenated Krebs bicarbonate solution and during this period the solutions were replaced every 15 min.

Contractile responses of femoral arterial strips to KCl, Bay K 8644 and noradrenaline

After the 90 min equilibration period, the femoral arterial strips were maximally activated by repeated administration (15 min) of 10^{-5} M noradrenaline (NA) until the responses were reproducible. Throughout the NA response, Krebs bicarbonate solution contained 3×10^{-7} M timolol to block β-adrenoceptor responses, because the β-adrenoceptor activities in these strips were significantly different in the SHR from those in WKY (Asano *et al.*, 1982).

Cumulative dose-response curves for the contractile effect of KCl (K^+) were determined by a stepwise increase in the concentration of K^+ as soon as a steady response to the preceding dose had been obtained. Following the determination of the response to K^+, the dose-response curve for the contractile effect of Bay K 8644 was determined in the same strip (Figure 1). Therefore, the contractile response to Bay K 8644 could be expressed as a percentage of the maximum contraction induced by K^+-depolarization (Asano *et al.*, 1984). Osmotic adjustment was not made when K^+ was added.

Cumulative dose-response curves for NA were determined in another series of experiments. After the 90 min equilibration period, the strips were maximally activated by repeated administration (30 min) of 60 mM K^+ until the responses were reproducible. The dose-response curve for NA was then determined in the presence of 3×10^{-7} M timolol to eliminate the possible β-adrenoceptor responses, as described above.

Dose-resonse curves for Ca^{2+} in K^+-depolarized strips were determined. In this experiment, strips were washed several times over 60 min with (nominally) Ca^{2+}-free K^+-depolarizing solutions (80 mM K^+ substitution for Na^+) (Asano & Hidaka, 1985) and a cumulative dose-response curve for Ca^{2+} was then determined. Dose-response curves for Ca^{2+} were also determined after the strips were washed for 60 min with Ca^{2+}-free Krebs bicarbonate solutions containing either 10^{-7} M Bay K 8644 or 10^{-5} M NA (plus 3×10^{-7} M timolol and 100 μg ml^{-1} ascorbic acid).

Effects of nifedipine on the contractile response to Bay K 8644

The effect of nifedipine on the dose-response curve of SHR femoral artery to Bay K 8644 was determined in the following way. Reproducibility of the dose-response curve for Bay K 8644 was first determined. In this experiment, following the determination of the dose-response curve for K^+, five dose-response curves

for Bay K 8644 were obtained from a single preparation with an interval of 80 min between each determination. Maximum contractions induced by Bay K 8644 were fairly constant throughout the five sequential dose-response curves. As the pD_2 values obtained from the second and subsequent dose-response curves were found to be identical, the second curve was taken as control and the effects of three concentrations of nifedipine were determined on the third, fourth and fifth curves.

The pA_2 value of nifedipine against Bay K 8644 was determined from the regression analysis of log (dose-ratio -1) against log [B]. Dose-ratio refers to the concentration of Bay K 8644 required to produce 50% of the maximum response (ED_{50}) in the presence of a concentration [B] of nifedipine, divided by the ED_{50} in the absence of nifedipine (Arunlakshana & Schild, 1959).

Effects of nifedipine on the dose-response curves to either Ca^{2+} (in K^+-depolarized strips) or NA were determined according to the method described previously (Asano & Hidaka, 1985).

Statistical analysis

Unless specified, results shown in the text and figures are expressed as the mean value ± s.e. (n = number of preparations, one preparation from each animal), and statistical significance was assessed using either Student's t test for paired data or that for unpaired data between two observations. Statistical significance was assumed when $P < 0.05$.

When assessing the ED_{50} value, responses to agonists were calculated as a percentage of the maximum response obtained with each agonist. The ED_{50} value was obtained visually from a plot of % response vs. log concentration of the agonist and expressed as the negative log (pD_2 value), or, for K^+, in mM.

Drugs and chemicals

The drugs used were Bay K 8644 (a kind gift from Drs S. Kazda and G. Franckowiak, Bayer AG, Wuppertal, FR Germany), nifedipine (Bayer Yakuhin Ltd, Osaka, Japan), phentolamine mesylate (Ciba-Geigy, Takarazuka, Japan), timolol maleate (Banyu Pharmaceutical Co., Tokyo, Japan) (−)-NA bitartrate (Sigma Chemical Co., St. Louis, MO) and papaverine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan). Bay K 8644 and nifedipine were dissolved in ethanol to make a stock solution of 10^{-3} M. These dihydropyridines were stored under refrigeration and protected from light. Aliquots of these solutions were then diluted with distilled water before use. To avoid adverse related effects, these dihydropyridines were used in concentrations below 10^{-6} M (the concentration of ethanol was below 0.1%). Phentolamine (10^{-2} M), timolol (10^{-2} M) and papaverine (10^{-2} M) was prepared in distilled water. NA was prepared daily in Krebs bicarbonate solution and kept on ice during the course of the experiment.

Results

Contractile responses of femoral arteries to K^+, Bay K 8644 and NA

Contractile responses of femoral arterial strips from SHR to exogenously added KCl (K^+) and Bay K 8644 are shown in Figure 1. The addition of K^+ in concentrations ranging from 3 to 60 mM elicited a

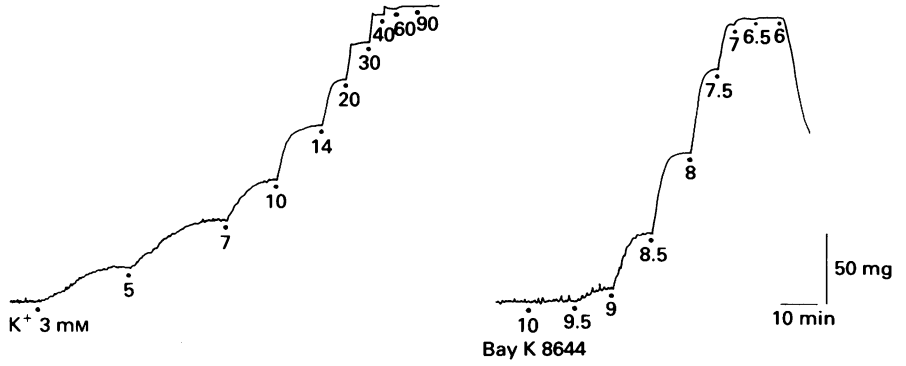

Figure 1 Typical recording of the responses to exogenously applied KCl (K^+) and Bay K 8644 in helical strips of femoral arteries isolated from 6 week old spontaneously hypertensive rats (SHR). Following the 90 min equilibration, repeated administration of 10^{-5} M noradrenaline (in the presence of 3×10^{-7} M timolol, a β-adrenoceptor antagonist) and subsequent 40 min washing, the dose-response curve for K^+ was determined. The dose-response curve for Bay K 8644 was then determined in the same strip after 80 min washing. The concentrations of Bay K 8644 are expressed as a negative log of the molar concentration.

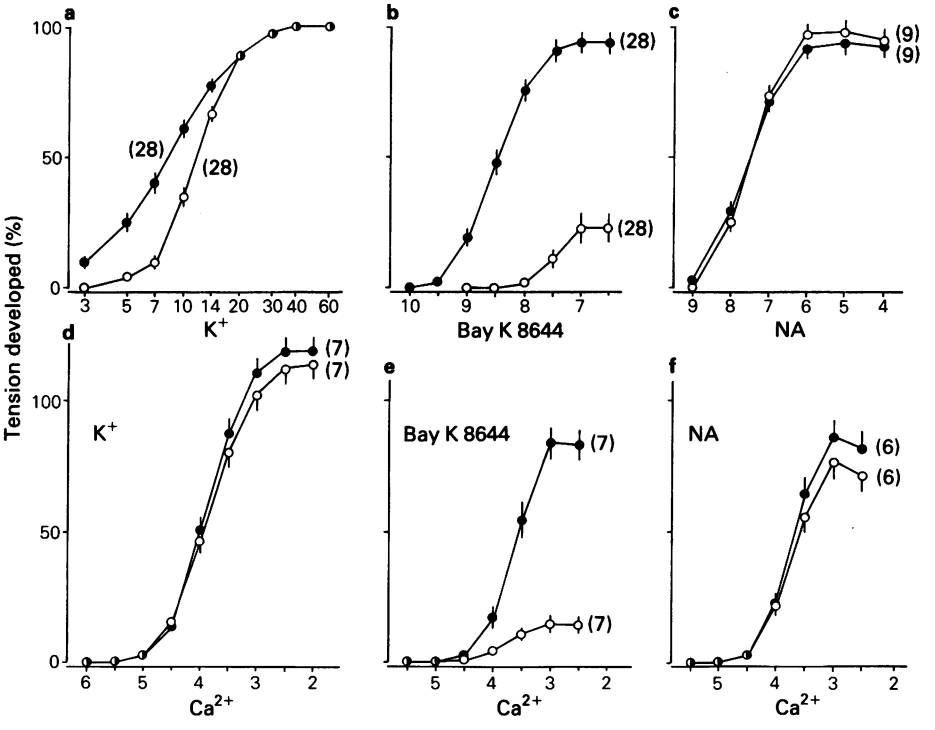

Figure 2 Cumulative dose-response curves for the contractile effects of KCl (a,d), Bay K 8644 (b,e) and noradrenaline (NA;c,f) in helical strips of femoral arteries isolated from 6 week old SHR (●) and age-matched WKY (○). (a) Dose-response curves for KCl (K^+) were determined by a stepwise increase in concentrations of K^+ as soon as a steady response to the preceding dose had been obtained. (b) Following the determination of the response to K^+ (as in a), the dose-response curve for Bay K 8644 was determined in the same strip. Experimental conditions were the same as in Figure 1. (c) Dose-response curves for noradrenaline were determined in another series of experiments in the presence of 3×10^{-7} M timolol. (d) Dose-response curves for Ca^{2+} in K^+-depolarized strips. Following the determination of 60 mM K^+ response (as in a), the strip was incubated in Ca^{2+}-free, K^+-rich Krebs bicarbonate solution (80 mM K^+ substitution for Na^+) for 60 min. (e) Dose-response curves for Ca^{2+} determined after incubation of the strip in Ca^{2+}-free Krebs bicarbonate solution containing 10^{-7} M Bay K 8644. Following the determination of the dose-response curve for Bay K 8644 in normal Krebs bicarbonate solution (as in b), the solution was replaced with a Ca^{2+}-free solution containing 10^{-7} M Bay K 8644. (f) Dose-response curves for Ca^{2+} determined after incubation of the strip in Ca^{2+}-free Krebs bicarbonate solution containing 10^{-5} M NA (plus 3×10^{-7} M timolol and 100 $\mu g\ ml^{-1}$ ascorbic acid). Following the determination of the dose-response curve for NA in normal Krebs bicarbonate solution (as in c), the solution was replaced with the Ca^{2+}-free solution containing NA. The concentrations of the agonists are expressed as a negative log of the molar concentration with the exception of K^+ where the concentration is expressed in mM. In all the panels, the response to 60 mM K^+ determined in the same strip was taken as 100%. Mean values of the maximum contractile tensions developed to 60 mM K^+ were 214 ± 20 mg (SHR, $n = 28$) and 233 ± 9 mg (WKY, $n = 28$, not significantly different from SHR), respectively. Vertical lines represent s.e. Numbers in parentheses indicate the number of preparations used.

dose-dependent contraction in strips of femoral arteries isolated from both SHR and WKY (Figure 2a). The strips from SHR femoral arteries had a lower threshold to K^+ than those from WKY arteries. The ED_{50} value of the response to K^+ in SHR strips (8.2 ± 0.6 mM, $n = 28$) was significantly ($P < 0.001$) lower than that in WKY strips (11.8 ± 0.5 mM, $n = 28$). Contractile responses to Bay K 8644 were quite different in SHR arterial strips from those of the WKY (Figure 2b). The addition of Bay K 8644 in concentrations ranging from 1×10^{-10} to 3×10^{-7} M elicited a dose-dependent contraction in strips of SHR femoral arteries. Maximum contraction was obtained when 1×10^{-7} M Bay K 8644 was added. A higher concentration of this agonist (1×10^{-6} M) produced a significant relaxation which may indicate a Ca^{2+} antagonistic effect (Figure 1). The threshold concentration of Bay K 8644 was significantly lower in SHR

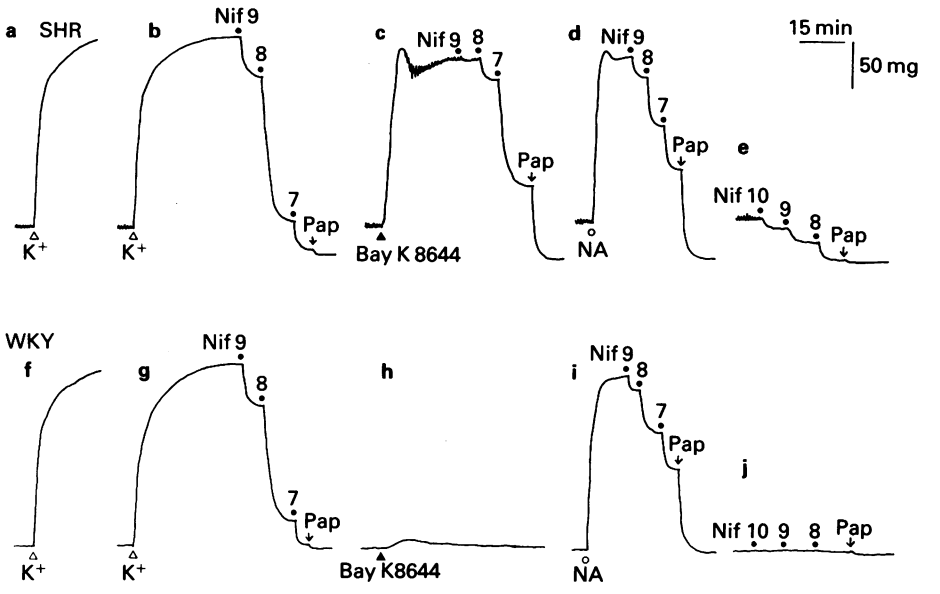

Figure 3 Relaxant responses to nifedipine in strips of femoral arteries isolated form 6 week old SHR (a–e) and age-matched WKY (f–j). Following the repeated application of 60 mM K^+, the strips were contracted with 60 mM K^+ (K^+), 10^{-7} M Bay K 8644 and 10^{-5} M noradrenaline plus 3×10^{-7} M timolol (NA). Nifedipine (Nif), 10^{-9} to 10^{-7} M (expressed as a negative log of the molar concentration), was added after the contraction of each agonist had reached a plateau. At the end of each experiment, papaverine (Pap) 10^{-4} M was added to obtain the maximum relaxation of the strip; (e) and (j) indicate the effects of nifedipine on the basal tone of strips. Note the significant relaxation induced by nifedipine in SHR strips.

than that in WKY (Figure 2b). The pD_2 value of the response to Bay K 8644 in SHR (8.55 ± 0.05, $n = 28$) was significantly ($P < 0.001$) different from the value in WKY (7.54 ± 0.06, $n = 28$). Maximum contraction by this agonist in SHR was approximately 4 times greater than the maximum in WKY. It is noteworthy that in SHR strips, maximum contraction induced by Bay K 8644 was the same as the maximum induced by K^+-depolarization. However, the contractile responses to NA via α-adrenoceptors in SHR femoral arterial strips were not significantly different from those in WKY strips (Figure 2c).

When the strips were fully depolarized by replacing the normal solutions with Ca^{2+}-free 80 mM K^+ solutions (Asano & Hidaka, 1985), there was no significant difference in the contractile responses to Ca^{2+} between the strips from SHR and WKY (Figure 2d). There was a significant difference in the contractile responses to Ca^{2+}, determined after incubation of the strips with Ca^{2+}-free solutions containing 10^{-7} M Bay K 8644, between the SHR and WKY (Figure 2e). Ca^{2+} influx through the α-adrenoceptor-operated channels, determined after incubation of the strips with Ca^{2+}-free solutions containing 10^{-5} M NA, in the SHR was not significantly different from that in the WKY (Figure 2f). Thus, the difference in the contractile responses to Ca^{2+} between the SHR and WKY can be seen only in the presence of Bay K 8644.

Effects of nifedipine on femoral arterial contraction

Vascular relaxant effects of nifedipine are shown in Figure 3. The addition of nifedipine to a strip precontracted with 60 mM K^+ produced a dose-dependent relaxation (Figure 3b,g). There was no significant difference in the extent of relaxation induced by nifedipine between the SHR and WKY (Figure 4a). However, in SHR strips, but not WKY strips, nifedipine induced relaxation responses below the resting tension, suggesting Ca^{2+} leakage to be the cause of the high resting tension in SHR (Figure 3b,g). This assumption is also indicated by the observation that nifedipine induced a relaxation response in strips from SHR at resting tension, but not in those from WKY (Figure 3e,j). Nifedipine also produced a dose-dependent relaxation in SHR strips precontracted with 10^{-7} M Bay K 8644 (Figure 3c), but the effective concentrations of nifedipine were much higher than those needed to relax K^+-contracted strips (Figure 3b,c and 4a,b). Bay K 8644 (1×10^{-7} M) was much less

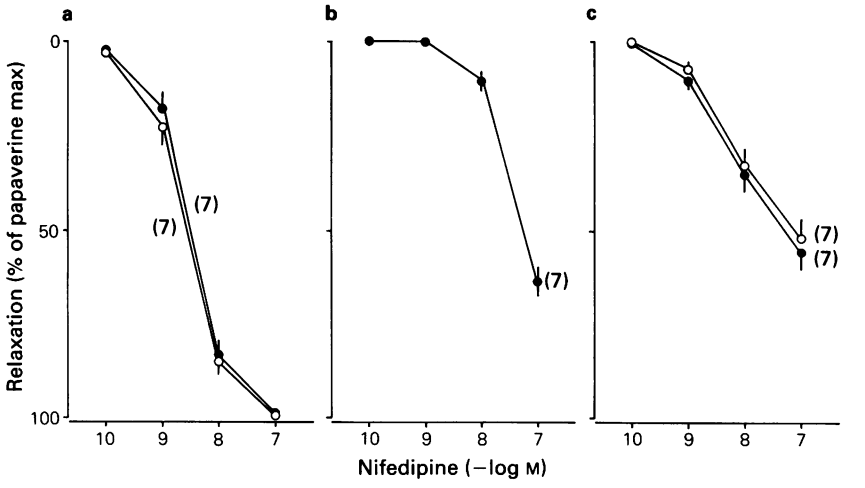

Figure 4 Dose-response curves for the relaxant effects of nifedipine in (a) KCl-, (b) Bay K 8644-, and (c) noradrenaline (NA)-contracted strips of femoral arteries isolated from 6 week old SHR (●) and age-matched WKY (○). Experimental conditions were the same as in Figure 3. Relaxation induced by 10^{-4} M papaverine was taken as 100%. The pD_2 values of nifedipine-induced relaxation in K^+-contracted strips (a) were 8.54 ± 0.07 (WKY, $n = 7$) and 8.51 ± 0.07 (SHR, $n = 7$, not significantly different from WKY), respectively. The pD_2 value of nifedipine-induced relaxation in Bay K 8644-contracted strips of SHR (b) was 7.26 ± 0.06 ($n = 7$, significantly different when compared to K^+-contracted strips of SHR, $P < 0.001$). The pD_2 values of nifedipine-induced relaxation in NA-contracted strips (c) were 7.11 ± 0.08 (WKY, $n = 7$) and 7.27 ± 0.08 (SHR, $n = 7$, not significantly different from WKY), respectively. Vertical lines represent s.e. Numbers in parentheses indicate the number of preparations used.

effective in eliciting contractile effects in WKY strips than in SHR strips (Figure 3h). There was no significant difference in nifedipine-induced relaxation of NA-induced contractions between the SHR and WKY (Figures 3d,i and 4c).

The type of antagonism induced by nifedipine was determined by investigating the effects of this antagonist on the dose-response curves of SHR strips to the contractile agonists (Figure 5). Nifedipine showed a competitive antagonism against responses to both Ca^{2+} (K^+-depolarization) and Bay K 8644, producing a rightward displacement of the dose-response curve for each agonist (Figure 5a and b). In contrast, the antagonism by nifedipine of the response to NA was a typical non-competitive type producing a reduction of the maximum response (Figure 5c). These results support the view proposed by Schramm *et al.* (1983a, b) that Bay K 8644 and nifedipine may act on a common site of the voltage-dependent Ca^{2+} channels. The Schild plot for nifedipine antagonism of the responses to Ca^{2+} (K^+-depolarization) gave a regression line with a slope of 1.06 and a pA_2 value of 9.42. The slope and pA_2 value of nifedipine antagonism of the responses to Bay K 8644 were 1.09 and 8.36, respectively (Figure 5d). Thus, the pA_2 value of nifedipine is much smaller for the antagonism of Bay K 8644 than for the antagonism of K^+-depolarization.

Dose-response curves of SHR strips to Bay K 8644 were not affected by either 1×10^{-6} M phentolamine, an α-adrenoceptor antagonist, or 3×10^{-7} M timolol. The pD_2 values of Bay K 8644 in the presence of phentolamine and of timolol were 8.43 ± 0.09 ($n = 4$) and 8.41 ± 0.12 ($n = 4$), respectively. These results suggest that the contractions induced by Bay K 8644 are not mediated through the release of endogenous NA or direct stimulation of α-adrenoceptors.

Discussion

The major conclusion in the present study is that in femoral arteries from SHR the voltage-dependent Ca^{2+} channels exist in an altered state. This is indicated from the following observations: (1) Bay K 8644, a Ca^{2+} channel agonist, elicited greater contractions in SHR femoral arteries than in WKY femoral arteries, (2) SHR femoral arteries were more sensitive to K^+-depolarization than WKY femoral arteries, (3) nifedipine, a Ca^{2+} channel antagonist, produced a relaxation from the resting tension only in SHR femoral arteries, and (4) once the femoral arteries were fully contracted with 60 mM K^+, nifedipine produced similar relaxations in the SHR and WKY. It is possible that the femoral artery from the SHR is depolarized

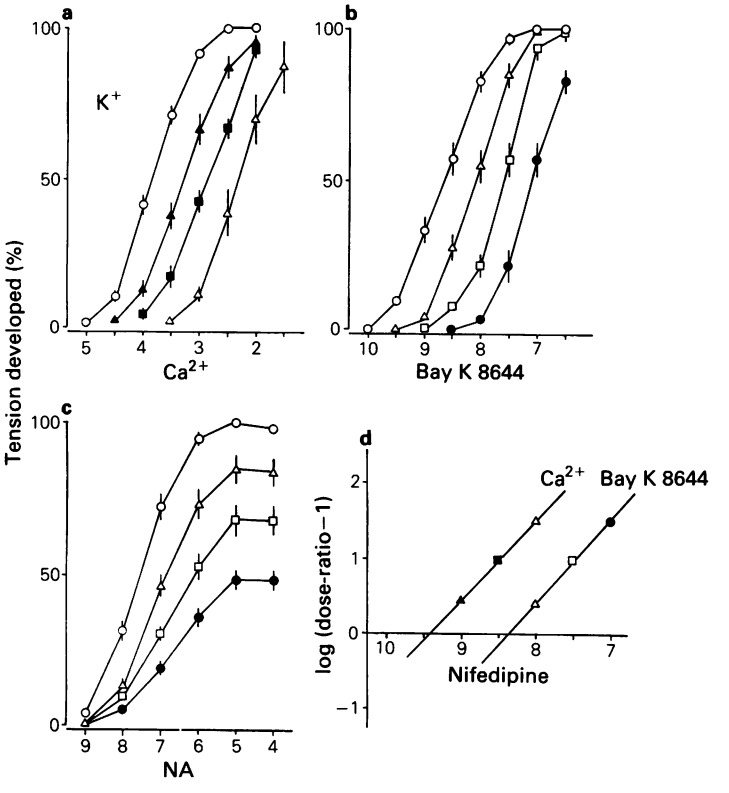

Figure 5 Effects of nifedipine on the dose-reponse curves for Ca^{2+} in K^+-depolarized strips (a) and for either Bay K 8644 (b) or noradrenaline (NA; c) in strips of femoral arteries isolated from 6 week old SHR. Effects of nifedipine were determined as described in Methods section. The concentrations of each agonist were expressed as a negative log of the molar concentration. Maximum contractile tension developed by each agonist in the absence of nifedipine (○) was taken as 100%. The concentrations of nifedipine used were 1×10^{-9} M (▲), 3×10^{-9} M (■), 1×10^{-8} M (△), 3×10^{-8} M (□) and 1×10^{-7} M (●). Note the competitive antagonism by nifedipine of the contractile responses to both Ca^{2+} (K^+-depolarized strip) and Bay K 8644. In contrast, nifedipine showed a typical non-competitive antagonism of the contractile response to NA. (d) Schild plot of the data. The pA_2 value of nifedipine was determined from the regression analysis of log (dose-ratio − 1) against log [B]. Dose-ratio refers to the ED_{50} of Bay K 8644 in the presence of a concentration of [B] of nifedipine, divided by the ED_{50} in the absence of nifedipine. The pA_2 value and slope for the antagonism by nifedipine of the Ca^{2+} response (K^+-depolarization) were 9.42 and 1.06, respectively, and those for the antagonism by nifedipine of the Bay K 8644 response were 8.36 and 1.09, respectively. Dose-response curves shown represent the mean, with vertical lines indicating s.e., of 6 to 7 preparations.

(compared with the WKY) as this would be expected to produce the changes that we observed.

Bay K 8644 produced a potent vasoconstriction in rat thoracic aorta (Mikkelsen *et al.*, 1985) and human umbilical artery (Goparakrishnan *et al.*, 1985), with a pD_2 value of 6.66 and 7.89, respectively. The maximum response of human umbilical artery to Bay K 8644 was the same as that induced by K^+-depolarization (Goparakrishnan *et al.*, 1985). In the present study on SHR femoral arteries, Bay K 8644 consistently induced a potent vasoconstrictor response under resting conditions (K^+ concentration 5.9 mM) with a pD_2 value of 8.55 and a maximum of 0.96 (expressed as a ratio to the maximum induced by K^+-depolarization). An extremely low concentration of Bay K 8644 (3×10^{-10} M) caused a contraction in SHR femoral arteries and the maximum contraction induced by this agonist was comparable to that induced by either K^+-depolarization or α-adrenoceptor stimulation. On the other hand, Bay K 8644 was less effective in eliciting a contraction in WKY femoral arteries. When the K^+ concentration in the bathing solution was elevated to 11.9 mM, WKY femoral arteries exhibited a potent contraction in response to Bay K 8644 which was

comparable to that induced by this agonist in SHR femoral arteries (data not shown). Therefore, the differences in the responses to Bay K 8644 between the SHR and WKY were not evident when the concentration of K^+ was elevated. The observations strongly suggest that in the SHR femoral artery, the voltage-dependent Ca^{2+} channels might be tonically activated and are directly activated by the Ca^{2+} channel agonist.

In rabbit aortic strips, Schramm *et al.* (1983a,b) have demonstrated that Bay K 8644 alone does not induce a contraction under resting conditions (K^+ concentration 2.6 mM), probably because this vessel is quiescent and therefore the voltage-dependent Ca^{2+} channels in this tissue under resting conditions are not activated. They demonstrated that this Ca^{2+} agonist lowered the threshold for the contractile response to exogeneously added K^+. Recent investigations using the rat tail artery (Su *et al.*, 1984), rabbit mesenteric artery (Kanmura *et al.*, 1984) and cat femoral artery (Salaices *et al.*, 1985) have also shown that Bay K 8644 does not elicit a mechanical response unless the K^+ concentration in the bathing solution is elevated. In strips of cat basilar arteries (Uski & Andersson, 1985), Bay K 8644 inconsistently induced a contraction, and in the presence of 10 mM K^+, it elicited a potent vasoconstriction with a pD_2 value of 8.68 and a maximum of 0.83 (expressed as a ratio to the contraction induced by 124 mM K^+). Similar results were obtained in cat middle cerebral arteries (Salaices *et al.*, 1985).

The assumption that the contractile responses of SHR femoral arteries to Bay K 8644 are exerted at voltage-dependent Ca^{2+} channels comes from the observation that the responses to this agonist are antagonized by a Ca^{2+} channel antagonist, nifedipine. The antagonism by nifedipine was competitive; it produced a rightward displacement of the dose-response curve of the SHR femoral artery to Bay K 8644. This supports the view proposed by Schramm *et al.* (1983a,b) that Bay K 8644 and nifedipine may act on a common dihydropyridine receptor regulating the Ca^{2+} influx through voltage-dependent Ca^{2+} channels.

Recent findings by Hess *et al.* (1984) suggest that dihydropyridine Ca^{2+} antagonists promote a mode of Ca^{2+} channel gating in which the channels are unavailable for opening, whereas dihydropyridine Ca^{2+} agonists induce a mode of gating where the channels exhibit long-lasting openings. The most likely explanation of the contractile activities of Bay K 8644 indicated in the present study is that the mechanism of action of Bay K 8644 would be to enhance the opening mode of the channels either by increasing the probability of openings or shortening the closed periods.

The Schild plot analysis for nifedipine antagonism of Bay K 8644 gave a pA_2 value of 8.36. This pA_2 value is significantly smaller than the pA_2 value (9.42) found for nifedipine antagonism of Ca^{2+}-induced contractions in K^+-depolarized strips in the present study and other smooth muscle preparations, in which the values range from 9.4 to 9.7 (Rosenberger *et al.*, 1979; Hashimoto *et al.*, 1979; Spedding, 1982; Su *et al.*, 1984). These results raise the possibility that the Ca^{2+} channels activated by Bay K 8644 are not the same as those activated by K^+-depolarization. The differences in the pA_2 values may suggest two distinct populations of Ca^{2+} channels or different states of the channels with a 10 fold difference in affinity for nifedipine. However, it is unlikely that nifedipine and Ca^{2+} are competing for the same binding site, yet their Schild plot is linear. It may be acceptable to use the pA_2 value as a convenient and readily understood measure of potency for nifedipine, but in its original form (Arunlakshana & Schild, 1959) it is only meaningful for a competitive antagonist. Moreover, experiments with Ca^{2+} (K^+-depolarization) and Bay K 8644 were conducted at different concentrations of K^+, so it may be inappropriate to compare the pA_2 value for Ca^{2+} with that for Bay K 8644, and to suggest that there may be two distinct populations of Ca^{2+} channels. Nifedipine may well compete with Bay K 8644 for its binding site. It is clearly shown in this study that the pA_2 value for nifedipine antagonism of the response to Bay K 8644 is 8.36.

Contractile effects of Bay K 8644 on the SHR femoral artery were not affected by α- and β-adrenoceptor antagonists, and thus are not due to the release of endogenous NA or direct stimulation of α-adrenoceptors. When a Ca^{2+}-free solution containing 10^{-4} M EGTA replaced the normal Krebs bicarbonate solution in the bath, Bay K 8644 failed to elicit a contraction. These results suggest that Bay K 8644 is a useful pharmacological tool for investigating the voltage-dependent Ca^{2+} influx in intact vascular smooth muscles. A combined study of the dihydropyridine Ca^{2+} agonist and antagonist (e.g. Bay K 8644 and nifedipine) is recommended to gain more insight into the nature of the regulatory mechanisms of voltage-dependent Ca^{2+} influx in intact smooth muscles. It has been suggested that Bay K 8644 is a partial Ca^{2+} agonist with Ca^{2+} antagonistic effects (Hess *et al.*, 1984, Su *et al.*, 1984). These antagonistic effects of Bay K 8644 were usually observed at higher concentrations (above 10^{-6} M). Also in this study, 10^{-6} M Bay K 8644 produced arterial relaxation which may indicate its Ca^{2+} antagonistic effects. Therefore, Ca^{2+} agonistic effects of Bay K 8644 were observed only over a relatively limited concentration range (below 10^{-6} M) in vascular smooth muscle.

In conclusion, Bay K 8644 appears to be a potent Ca^{2+} agonist in strips of SHR femoral arteries, and thus a useful pharmacological tool for investigating the voltage-dependent Ca^{2+} influx of vascular smooth

muscle. The competitive antagonism observed between Bay K 8644 and nifedipine clearly suggests that Bay K 8644 acts primarily on the same site as nifedipine, presumably the voltage-dependent Ca^{2+} channel. Schild plot analysis clearly demonstrated a different pA_2 value of nifedipine antagonism against Bay K 8644 from that against K^+-depolarization, suggesting that the state of voltage-dependent Ca^{2+} channels may differ according to whether they are stimulated by Bay K 8644 or K^+-depolarization. Whether or not the Ca^{2+} channels activated by Bay K 8644 are identical to or in the same state as those activated by K^+-depolarization is the subject of current investigations.

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