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Role of the endothelial system in BAY K 8644 enantiomer and nifedipine vasomodulator action in rat aorta

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The potential importance of the endothelial system in regulating the effects of (–)-Bay K 8644 (0.1 μ M), (+)-Bay K 8644 (0.1 μ M) and nifedipine (10 nM) on resting tension, on contractile responses to noradrenaline (NA) and Ca^{2+} (in a Ca^{2+} -free high- K^+ solution), and on basal, NA-induced and K^+ -induced $^{45}\text{Ca}^{2+}$ uptake, was investigated in rat aorta rings. Mechanical removal of endothelium considerably potentiated the contractile response induced by NA in standard medium and by Ca^{2+} in Ca^{2+} -free high- K^+ (15 mM) medium, but did not modify the response induced by Ca^{2+} in Ca^{2+} -free high- K^+ (55 mM) medium or by NA in Ca^{2+} -free medium. Furthermore, the basal $^{45}\text{Ca}^{2+}$ uptake and that induced by NA (10 μ M) or KCl (15 and 55 mM) were similar in endothelium-rubbed and intact rings. (–)-Bay K 8644 (0.1 μ M) shifted the NA and Ca^{2+} concentration-response curves to the left with potentiation of the maximal contraction. However, (+)-Bay K 8644 (0.1 μ M) and nifedipine (10 nM) caused a shift to the right, with depression of the maximal contraction. The NA concentration-response curves, and those of Ca^{2+} in Ca^{2+} -free high- K^+ (55 mM) medium, were affected by the drugs to similar extents, and were not modified by the presence or absence of endothelial cells. The drugs tested did not affect resting tension. Basal $^{45}\text{Ca}^{2+}$ uptake was not modified by either nifedipine or the Bay K 8644 enantiomers. On the other hand, (–)-Bay K 8644 increased with equal effectiveness both NA- and KCl-induced $^{45}\text{Ca}^{2+}$ uptake, whilst (+)-Bay K 8644 and nifedipine inhibited both uptakes. The presence or absence of endothelium did not modify these effects. These results suggest that, in rat aorta, the endothelial system does not modulate either the agonist effect of (–)-Bay K 8644 or the antagonistic effects of (+)-Bay K 8644 and nifedipine. Furthermore, our data indicate that the effects of Bay K 8644 enantiomers and nifedipine on the contractile responses and $^{45}\text{Ca}^{2+}$ uptake elicited by NA and high- K^+ (55 mM) solutions are similar.

Aorta (rat); Endothelium; BAY k 8644 enantiomers; Nifedipine; $^{45}\text{Ca}^{2+}$ influx

1. Introduction

Transmembrane influx of extracellular Ca^{2+} through specific Ca^{2+} channels is recognized to be of importance in the excitation-contraction coupling of vascular smooth muscle cells. The discovery of the existence of Ca^{2+} channels (receptor-operated channels (ROCs) and potential-dependent channels (PDCs)) has allowed evaluation of the function of Ca^{2+} in this coupling (Putney, 1987; Bolton et al., 1988). Ca^{2+} entry may be inhibited by Ca^{2+} channel antagonists (Cauvin et al., 1983) or facilitated by Ca^{2+} channel agonists such as the nifedipine analogue, Bay K 8644 (Schramm et al., 1983). Franckowiak et al. (1985) have shown that the (–) enantiomer of Bay K 8644 has the Ca^{2+} channel

agonistic properties of the racemic compound whereas (+)-Bay K 8644 exhibits Ca^{2+} channel antagonistic properties similar to those of nifedipine.

Some authors (Meisheri et al., 1981; Yamamoto et al., 1984) have reported that nifedipine and Bay K 8644 interfere mainly with PDCs; this provides pharmacological evidence in favour of the existence of distinct populations of ROCs and PDCs. Other authors (Chiu et al., 1986; Dong and Wadsworth, 1986), however, have reported that these drugs act with similar effectiveness on both PDCs and ROCs, suggesting that the two types of Ca^{2+} channel have similar structural characteristics.

Since the early studies of Furchgott and Zawadzki (1980), the importance of the endothelial system in modulating the effects of several drugs on vascular smooth muscle tone has been abundantly demonstrated. It is known that endothelial cells are active and regulate vascular smooth muscle tone by releasing relaxing and contracting substances in response to recep-

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for activation or to physiological stimuli (Angus and Locks, 1989). The factors released include a variety of compounds, one of which referred to as endothelium-derived relaxing factor (EDRF), has been identified as nitric oxide (Ignarro et al., 1987; Palmer et al., 1987). EDRF activates soluble guanylate cyclase, leading to an increase in guanosine 3',5'-cyclic monophosphate (cyclic GMP) in vascular smooth muscle (Ignarro and Kadowitz, 1985).

The effects of Ca^{2+} channel modulators on the release of EDRF from endothelial cells are not clear. Rubanyi et al. (1985) reported that (\pm)-Bay K 8644 potentiates the release of EDRF; Singer and Peach (1982) found that Ca^{2+} channel blockers inhibit its release, whereas several other workers (Spedding et al., 1986; Kikkawa et al., 1989) have concluded that Ca^{2+} channel modulators do not modify the release of EDRF from endothelial cells at all.

In view of these conflicting reports, the present study considers for the first time the involvement of the endothelial system in the regulation of the vasomodulator effects of Bay K 8644 enantiomers in rat aorta.

2. Materials and methods

2.1. Tissue preparation

Female Sprague-Dawley rats weighing 350–400 g were killed by stunning and exsanguination. The thoracic aorta was rapidly removed, placed in a Petri dish with Tris solution (composition mM: NaCl 134.8; KCl 4.7; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2; Tris 5; HCl 5; glucose 10.1), cleaned of connective tissue, stripped (if necessary) of endothelium by rubbing the intimal surface with a wooden rod, and cut into 4-mm long cylindrical segments of approximately 5–10 mg. In some preparations, a simple haematoxylin-eosin staining technique was used to verify the absence of endothelial cells and the integrity of the underlying smooth muscle.

2.2. Contraction studies

General procedure: aorta rings were immediately transferred to an organ bath containing 20 ml of the above Tris solution, with temperature held at 37°C and bubbled with 100% O_2 . Two stainless steel pins were inserted through the lumen of each segment: one pin was fixed to the organ bath and the other was connected to a CPOL force-displacement transducer for recording of isometric tension using a computerized Celaster IOS 1 system. The preparations were equilibrated at a resting tension of 1 g for at least 1 h, during which time the physiological solution was replaced every 10 min. The presence or absence of endothelium was verified by evaluating the effects of 1 μM acetyl-

choline and 10 μM methylene blue on maximal contraction in response to vasoconstrictor agents.

NA concentration-response curves: cumulative concentration-response curves were obtained using Van Rossum's method (Van Rossum, 1963), in which progressively higher concentrations are applied when a steady state level has been reached for the preceding concentration. Initially, two reproducible control concentration-response curves were obtained with a 60-min delay between the two, to allow for washout and to minimize the possibility of receptor desensitization. The tissues were then incubated with nifedipine or Bay K 8644 enantiomers for 20 min and a third curve was obtained.

Ca^{2+} concentration-response curves: after an equilibration period of 1 h in normal Tris solution, tissues were incubated for 30 min in Ca^{2+} -free depolarizing Tris solution containing 15 or 55 mM of KCl instead of the equivalent amount of NaCl, in order to maintain osmolarity. Calcium chloride (10 μM –10 mM) was then added to the bath in stepwise fashion. After two reproducible control concentration-response curves had been obtained, the tissues were incubated as above with nifedipine or Bay K 8644 enantiomers for 20 min and a third curve was then obtained.

Studies in Ca^{2+} -free medium: aorta preparations were equilibrated for 60 min in normal Tris solution then washed four times over a 20-min period with a Ca^{2+} -free solution (containing 0.5 mM EGTA); contraction was then elicited with noradrenaline (NA; 10 μM). To evaluate the effects of the drugs under study, the preparations were washed again in normal Tris solution for 60 min (to fill the Ca^{2+} stores depleted by the first contraction). There followed a further 20-min preincubation in Ca^{2+} -free solution before a suitable concentration of (–)-Bay K 8644, (+)-Bay K 8644 or nifedipine was added, followed 10 min later by NA (10 μM). Identically treated aorta rings were simultaneously subjected to the same procedure without addition of drugs.

2.3. $^{45}\text{Ca}^{2+}$ influx

Aorta rings were equilibrated for at least 60 min in normal HEPES physiological solution (composition mM: NaCl 139; KCl 5; MgCl_2 1; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1.5; HEPES 5; glucose 10) maintained at 37°C and bubbled with 100% O_2 . Following equilibration, the tissues were first incubated for 5 min in a $^{45}\text{Ca}^{2+}$ (New England Nuclear, specific activity 35 mCi/mg)-containing medium (0.6 $\mu\text{Ci}/\text{ml}$) then for 2 min in the same solution with or without NA (10 μM), or for 5 min in a high- K^+ HEPES solution (15 or 55 mM), to evaluate the effects of these vasoconstrictor agents on $^{45}\text{Ca}^{2+}$ uptake.

To investigate the effects of (–)-Bay K 8644, (+)-Bay K 8644 and nifedipine on this uptake, the drugs

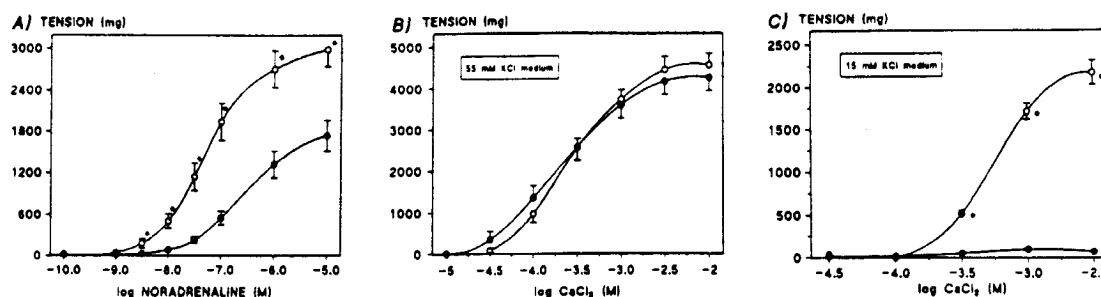


Fig. 1. Effects of endothelium-stripping (●, rings with endothelium; ○, rings without endothelium) on (A) cumulative NA concentration-response curves in medium with normal Ca²⁺; and on cumulative CaCl₂ concentration-response curves in (B) 55 mM KCl medium, and (C) in 15 mM KCl medium. Each point represents the mean response of at least 10 rat aorta rings. S.E.M.s are represented by vertical lines (except where the standard error was smaller than the point representing the mean); * significant difference ($P \leq 0.05$).

were added to the bath 20 min before, and during, the incubation period with ⁴⁵Ca²⁺. Thereafter, the preparations were washed for 5 min in 500 ml of La³⁺ solution (composition mM: NaCl 118; KCl 6; Tris 5.4; MgSO₄ · 7H₂O 1.2; LaCl₃ · 7H₂O 50; glucose 11) (pH = 6.8) bubbled with 95% O₂ and 5% CO₂ in order to remove extracellular Ca²⁺ from the tissue. The aorta rings were then blotted, weighed and digested in 1 ml H₂O₂ (110 volumes) at 115°C for 90 min. After cooling, 5 ml of Ready-Safe Beckman was added and the radioactivity of the samples was measured in a liquid scintillation counter (Beckman LS 3801).

2.4. Expression and statistical analysis of results

Unless otherwise specified, the results shown in the text and figures are expressed as means ± S.E.M. The statistical significance of differences between two means ($P \leq 0.05$ or $P \leq 0.01$) was determined using Student's two-tailed t-test for paired and unpaired data.

Contractile responses to NA and CaCl₂ are expressed as percentages of the maximal contraction ($E_{max} = 100\%$) reached in the concentration-response curves obtained before incubation with the Ca²⁺ channel modulators. Using linear regression (least squares method), pD₂ values (the negative logarithm of the

molar concentration of the agonist required to elicit 50% of maximal response) were obtained for the vasoconstrictor agents.

⁴⁵Ca²⁺ uptake by the tissues was calculated using the formula: uptake (nmol ⁴⁵Ca²⁺/kg wet tissue) = (scintillation count per min in tissue/wet tissue weight kg) × (nmol ⁴⁵Ca²⁺ in 1 l of medium/scintillation count per min in 1 l of medium). Note that the numerator of the second factor in this expression is the concentration of ⁴⁵Ca²⁺, not the total Ca²⁺ concentration. The difference in ⁴⁵Ca²⁺ content between NA- and KCl-stimulated rings and non-stimulated rings was defined as 100%, and the effects of the drugs studied are thus expressed as a percentage of this maximum difference.

2.5. Drugs and chemicals

The following drugs were used: Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate) enantiomers and nifedipine (all three from Bayer); (–)-noradrenaline bitartrate, acetylcholine chloride and methylene blue (all from Sigma).

Bay K 8644 enantiomers and nifedipine were dissolved in 95% ethanol to make 10 mM stock solutions, and aliquots of these solutions were then diluted with de-ionized water. NA and acetylcholine were prepared

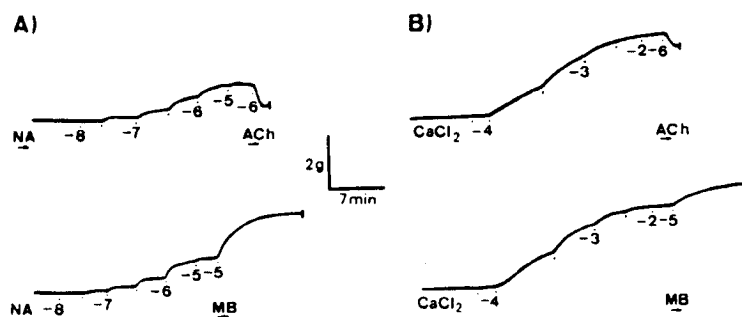


Fig. 2. Rat aorta rings with intact endothelium: typical effects of acetylcholine (ACh) and methylene blue (MB) on (A) contractile responses to noradrenaline (NA) and (B) CaCl₂ in a Ca²⁺-free high-K⁺ (55 mM) solution. The different concentrations are expressed as logarithms of molar concentrations. The traces have been retouched for clarity.

daily with de-ionized water from a stock solution (100 mM) kept at -20°C . Sodium bisulphite (0.2%) was added to the NA stock solution to prevent oxidation. Due to the photosensitivity of the dihydropyridines, all experiments with these drugs were carried out in the dark. All reagents used in the preparation of physiological solutions were of analytical grade.

3. Results

3.1. Effects of endothelium on mechanical responses

NA elicited concentration-dependent contractions in endothelium-rubbed and intact aorta rings. Mechanical removal of endothelium shifted the NA concentration-response curves considerably to the left with potentiation of the maximal contraction (E_{\max}) (fig. 1A). The pD_2 values obtained were 6.3 ± 0.09 (with endothelium) and 6.7 ± 0.13 (without endothelium) ($n = 17$, $P \leq 0.01$). Once E_{\max} had been reached, the addition of acetylcholine ($1 \mu\text{M}$) to intact rings induced a pronounced relaxation ($69 \pm 2.4\%$, $n = 18$, $P \leq 0.01$), whilst methylene blue ($10 \mu\text{M}$) induced a potentiation of $183 \pm 8.4\%$ ($n = 8$, $P \leq 0.01$) (fig. 2A). Neither acetylcholine nor methylene blue had significant effects on the NA-induced E_{\max} in endothelium-rubbed rings.

When endothelium-rubbed and intact rings were exposed to Ca^{2+} -free high- K^+ (55 mM) solution, addition of Ca^{2+} produced a concentration-dependent response. The pD_2 values were 3.58 ± 0.119 and 3.59 ± 0.046 ($n = 17$, $P > 0.05$). The presence or absence of endothelium did not alter the contractile effect induced by Ca^{2+} in a Ca^{2+} -free high- K^+ 55 mM solution (fig. 1B). In intact rings, acetylcholine ($1 \mu\text{M}$) and methylene blue ($10 \mu\text{M}$) induced, respectively, relaxation ($20 \pm 4.1\%$, $n = 14$, $P \leq 0.01$) and potentiation ($35 \pm 8.1\%$, $n = 6$, $P \leq 0.01$) of the Ca^{2+} E_{\max} (fig. 2B); these responses were significantly different ($P \leq 0.01$) from those observed in the NA concentration-response curve. Neither acetylcholine nor methylene blue had significant effects on the Ca^{2+} -induced E_{\max} in endothelium-rubbed rings.

The contractile effect on intact rings of Ca^{2+} in a Ca^{2+} -free high- K^+ (15 mM) solution was negligible whilst in endothelium-rubbed rings Ca^{2+} produced concentration-dependent contractions (fig. 1C).

In Ca^{2+} -free medium, NA ($10 \mu\text{M}$) produced a characteristic contraction with two distinct components: an initial transient contraction (fast component) that relaxed to a sustained tension (slow component) (table 1). The differences between contractions obtained in endothelium-rubbed and intact rings were not significant (table 1).

3.2. Effects of endothelium on $^{45}\text{Ca}^{2+}$ influx

$^{45}\text{Ca}^{2+}$ uptake by aorta segments in the absence of other agents (basal uptake) was 8.2 ± 0.18 nmol/kg wet tissue ($n = 30$) in intact preparations, and 8.7 ± 0.20 nmol/kg wet tissue ($n = 29$) in endothelium-rubbed preparations. The difference between the two means was not significant.

NA ($10 \mu\text{M}$) significantly increased ($P \leq 0.01$) basal $^{45}\text{Ca}^{2+}$ uptake in both endothelium-rubbed and intact rings ($^{45}\text{Ca}^{2+}$ tissue content: 10.4 ± 0.18 nmol/kg ($n = 12$) with endothelium, and 11.3 ± 0.20 nmol/kg ($n = 12$) without endothelium). High K^+ (15 and 55 mM) also induced a significant ($P \leq 0.01$) increase in basal uptake in both endothelium-rubbed and intact rings. The $^{45}\text{Ca}^{2+}$ tissue content in 15 mM K^+ medium was 11.9 ± 0.41 nmol/kg ($n = 10$) with endothelium and 13.3 ± 0.58 nmol/kg ($n = 8$) without endothelium; in 55 mM K^+ medium, the corresponding values were 10.8 ± 0.41 nmol/kg ($n = 7$) with endothelium, and 11.0 ± 0.37 nmol/kg ($n = 9$) without endothelium.

Mechanical removal of the endothelium did not significantly affect the $^{45}\text{Ca}^{2+}$ influx elicited by either K^+ or NA.

3.3. Effects of Bay K 8644 enantiomers and nifedipine on mechanical responses

Rat aorta rings lack spontaneous activity. Resting tension was not affected by either Bay K 8644 enantiomers ($0.1 \mu\text{M}$) or by nifedipine (10 nM) (data not shown). However, although Ca^{2+} (in Ca^{2+} -free high- K^+

TABLE 1

Rat aorta rings without endothelium: effects of the drugs tested on noradrenaline (NA; $10 \mu\text{M}$)-induced contractile responses (mg) in Ca^{2+} -free medium. Contractions were recorded following 10-min exposure to the drug under study or to the corresponding control solution. The values are presented as means \pm S.E. No significant differences were observed between treated and control preparations.

Compound	n	Fast component (mg)	Slow component (mg)
NA	10	1137 ± 96.4	276 ± 35.0
NA + (–)-Bay K 8644 ($0.1 \mu\text{M}$)	5	1088 ± 118.5	278 ± 35.1
NA + (+)-Bay K 8644 ($0.1 \mu\text{M}$)	5	1019 ± 94.8	216 ± 30.5
NA + nifedipine (10 nM)	5	1035 ± 125.3	236 ± 35.9

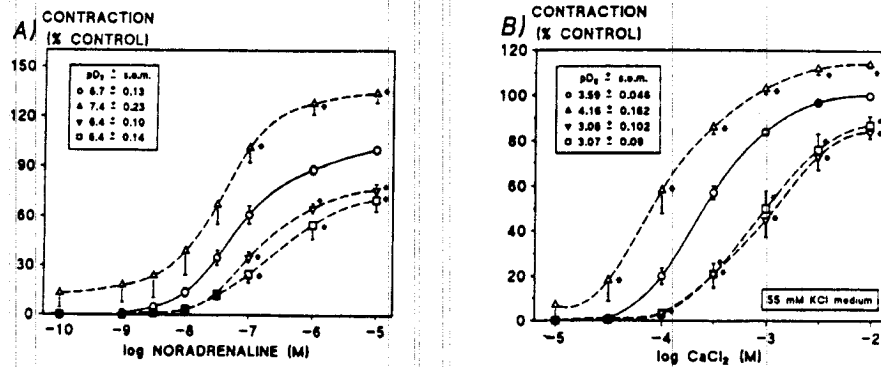


Fig. 3. NA concentration-response curves (A) and CaCl_2 -induced concentration-response curves in KCl 55 mM depolarizing medium (B), in the absence (control) or presence of the drugs under study (○, control; △, (–)-Bay K 8644 (0.1 μM); ▽, (+)-Bay K 8644 (0.1 μM); □, nifedipine (10 nM)), in endothelium-rubbed aorta rings. The treated tissues were preincubated for 20 min with the drug concerned prior to making the concentration-response curve. For each ring, the maximal contraction induced by NA or CaCl_2 prior to addition of the drug was considered to be the full contraction (100%). Each point represents the mean response of at least five rings \pm S.E. (vertical lines); * significant difference with respect to control ($P \leq 0.05$).

15 mM solution) did not induce contraction in untreated intact rings, it did cause concentration-dependent contraction in intact rings preincubated with (–)-Bay K 8644, with a maximal tension of 2532 ± 504 mg and a pD_2 of 3.55 ± 0.05 being obtained.

(–)-Bay K 8644 (0.1 μM) shifted the NA concentration-response curve to the left in both endothelium-rubbed and intact preparations, with potentiation of the maximal contraction, whereas (+)-Bay K 8644 and nifedipine non-competitively antagonized NA-induced contractions, shifting the concentration-response curves to the right, with depression of the maximal contraction (fig. 3A). The presence of endothelium did not modify the effects of the drugs on the concentration-response curves.

As in the concentration-response curves for NA, (–)-Bay K 8644 (0.1 μM) shifted the Ca^{2+} concentration-response curve to the left with potentiation of the maximal contraction, (+)-Bay K 8644 (0.1 μM) and nifedipine (10 nM) shifted the concentration-response curves to the right (fig. 3B). The effects of the drugs were not modified by the presence of endothelial cells.

In endothelium-rubbed rings, (–)-Bay K 8644 (0.1 μM) significantly enhanced the contractile responses induced by Ca^{2+} (in a Ca^{2+} -free high- K^+ 15 mM

solution), whereas (+)-Bay K 8644 (0.1 μM) and nifedipine (10 nM) inhibited them. The effects of (–)-Bay K 8644 were greater in 15 mM KCl than in 55 mM, whereas the effects of (+)-Bay K 8644 and nifedipine, in terms of extent of the concentration curve shift, were greater in 55 mM KCl.

In Ca^{2+} -free medium, the Bay K 8644 enantiomers (0.1 μM) and nifedipine (1 nM) did not alter the contractile effect induced by NA in either intact or endothelium-rubbed rings; thus the presence of endothelium did not modify the effects of the drugs (table 1).

3.4. Effects of Bay K 8644 enantiomers and nifedipine on $^{45}\text{Ca}^{2+}$ influx

The addition of Bay K 8644 enantiomers (1 μM) or nifedipine (10 nM) did not affect basal $^{45}\text{Ca}^{2+}$ uptake (data not shown).

(–)-Bay K 8644 (0.1 μM) potentiated the $^{45}\text{Ca}^{2+}$ influx induced by NA and K^+ with equal effectiveness, whereas (+)-Bay K 8644 and nifedipine inhibited it, again with equal effectiveness (table 2). The presence of endothelium did not alter the effects of the drugs tested.

TABLE 2

Rat aorta rings without endothelium: effects of the drugs studied on noradrenaline- and KCl-induced $^{45}\text{Ca}^{2+}$ uptake, expressed as percentage modification of the response in the absence of the drug (see Materials and methods). Treated tissues were preincubated for 20 min with the drug concerned, then for 5 min with the drug and 0.6 $\mu\text{Ci}/\text{ml}$ of $^{45}\text{Ca}^{2+}$, prior to exposure for 2 min to noradrenaline or 5 min to KCl (55 or 15 mM). No significant differences were observed between noradrenaline- and 55 mM KCl-induced uptake.

Compound	Noradrenaline (10 μM)	KCl (55 mM)	KCl (15 mM)
(–)-Bay K 8644 (0.1 μM)	114 ± 13.6 (n = 7)	100 ± 13.1 (n = 6)	171 ± 28.4 (n = 8)
(+)-Bay K 8644 (0.1 μM)	-22 ± 8.9 (n = 6)	-28 ± 5.2 (n = 5)	-32 ± 6.3 (n = 6)
Nifedipine (10 nM)	-84 ± 11.3 (n = 7)	-80 ± 7.3 (n = 6)	-61 ± 13 (n = 6)

4. Discussion

Mechanical removal of the endothelium considerably potentiated the contractile effect induced by NA, as had been found earlier (Malta et al., 1986; Martin et al., 1986; Godfraind, 1986) and contrary to the results obtained by Lai et al. (1989). Possibly, the different Ca^{2+} concentrations of the physiological solutions (López-Jaramillo et al., 1990) and the different techniques used for endothelium removal explain the variability of the results reported. Although NA might induce the release of EDRF through stimulation of α_2 -adrenoceptors (which, according to Eglème et al. (1984), may be present on the surface of rat aorta endothelial cells), it seems more probable that the endothelium-dependent inhibitory effects observed are largely due to spontaneous release of EDRF (Martin et al., 1986).

The presence of endothelium did not modify the contractile effect induced by Ca^{2+} (in Ca^{2+} -free high- K^+ 55 mM solution), in accordance with the results of previous studies (Lai et al., 1989; Godfraind, 1986). This result is confirmed by the fact that the release of EDRF from endothelial cells is a Ca^{2+} -dependent process which is however not modulated by Ca^{2+} influx at high K^+ , as voltage-dependent Ca^{2+} channels are not present in these cells (see Angus and Cocks, 1989 for review). However, the modulatory effect of endothelium is very marked in aorta rings depolarized with 15 mM K^+ (similar effects were reported by Spedding et al. (1986)). This difference is possibly due to the spontaneous release of EDRF having a more significant effect on partial contractions, such as those induced by 15 mM K^+ , than on full contractions, such as those induced by 55 mM K^+ .

The results obtained in Ca^{2+} -free medium indicate that mechanical removal of rat aorta endothelium does not modify NA-induced contractile effects, perhaps due to the fact that EDRF release is a Ca^{2+} -dependent process (again see Angus and Cocks, 1989 for a review).

The presence of endothelium did not modify either basal $^{45}\text{Ca}^{2+}$ uptake or the uptake induced by NA and K^+ (15 and 55 mM). Other authors have proposed that EDRF, through increasing cyclic GMP, mediates a reduction in Ca^{2+} influx via ROCs but not via PDCs (Godfraind, 1986). It is possible that, under certain specific conditions, ROC gating is sensitive to the presence of endothelium but, consistent with the results of Malta et al. (1986), our results show that a reduction in Ca^{2+} influx cannot be the sole explanation for the modulatory effect of endothelium on NA and 15 mM K^+ contractions. Others mechanisms, such as a reduction in the release of intracellular Ca^{2+} or activation of Ca^{2+} removal mechanisms such as Ca^{2+} ATPase may be implicated (see, for example, Cornwell and Lincoln, 1989). However, the possibility cannot be

ruled out that a greater number of experimental replications might reveal some differential effects of endothelium.

In the present study, neither the Bay K 8644 enantiomers nor nifedipine modified basal tension, whereas (–)-Bay K 8644 elicited contractile responses in aorta rings when these had been partially depolarized with 15 mM K^+ . This suggests that Ca^{2+} channel-modulators do not affect Ca^{2+} leak channels (passive Ca^{2+} channels), and that other types of Ca^{2+} channels are not operative under resting conditions. The need for partial depolarization for Ca^{2+} channel agonists ((±)-Bay K 8644) to elicit contraction has been observed in some vascular preparations but not in others (see Alonso et al., 1989 and Wanstall and O'Donnell, 1989 for a review). Such apparently conflicting results might be due to different resting membrane potentials in the various tissues studied (Asano et al., 1987; Wanstall and O'Donnell, 1989).

It has been shown that activation by noradrenaline of α_1 -adrenoceptors in rat aorta induces a two-phase contraction: an initial transient contraction caused by the inositol 1,4,5-trisphosphate-mediated release of Ca^{2+} from the intracellular stores, and a slow, sustained contraction caused by Ca^{2+} influx through the receptor-operated Ca^{2+} channels (Putney, 1987; Zelis and Moore, 1989). If it is borne in mind that the data obtained from the concentration-response curves and the $^{45}\text{Ca}^{2+}$ influx experiments provide information about the effect of the drugs on sustained contractions, our results show that (–)-Bay K 8644 activates receptor-operated Ca^{2+} channels and that (+)-Bay K 8644 (weakly) and nifedipine (strongly) inhibit them. Such results are not in accordance with early reports of the actions of (±)-Bay K 8644 (Yamamoto et al., 1984) and nifedipine (Meisheri et al., 1981); they do, however, confirm the results of several more recent studies on (±)-Bay K 8644 (Dong and Wadsworth, 1986; Babich et al., 1989) and nifedipine (Godfraind, 1983; Orallo et al., 1991). This suggests that there is an active dihydropyridine receptor in receptor-operated channels, unless it is additionally supposed that NA acts to facilitate the opening of potential-dependent channels. Whether or not depolarization initiates contraction is however debatable, despite the numerous studies which have tried to answer this question; the most widely held view (see, for example, Garland, 1989) is that NA-induced depolarization plays an important but not essential role in contraction.

It has been reported that high K^+ concentrations cause marked contractions in rat aortic tissue by depolarizing smooth muscle cells and increasing the influx of Ca^{2+} through L potential-dependent channels (Bolton et al., 1988). As was the case with noradrenaline-induced contractions and NA-induced $^{45}\text{Ca}^{2+}$ uptake, (–)-Bay K 8644 potentiated Ca^{2+} -induced contractions

(in Ca^{2+} -free high- K^+ 55 mM solution) and KCl-induced $^{45}\text{Ca}^{2+}$ uptake, whereas nifedipine and (+)-Bay K 8644 inhibited them. This indicates that the dihydropyridines also act on potential-dependent channels (Franczkowiak et al., 1985; Godfraind, 1983).

The vascular effects of (-)-Bay K 8644 were greater in 15 mM (enhancing the open state of L channels) than in 55 mM KCl; nifedipine and (+)-Bay K 8644, on the other hand, were more effective in 55 mM KCl (increasing the proportion of inactivated channels). These results are in accordance with the hypothesis that the interaction of Ca^{2+} channel modulators with L channels can be described by a modulated receptor model (Wibo, 1989).

The presence of endothelial cells did not modify the effects of Bay K 8644 enantiomers and nifedipine on contraction and $^{45}\text{Ca}^{2+}$ influx, suggesting that Ca^{2+} channel modulators do not affect the release of EDRF. Rubanyi et al. (1985) reported that (\pm)-Bay K 8644 stimulates EDRF release; if we accept this premise, the potentiating effects of (-)-Bay K 8644 should be less marked in intact preparations than in endothelium-rubbed preparations as a result of the vasodilator action of the EDRF released by the endothelial cells in response to the presence of the drug. As this was clearly not the case, our results suggests that (-)-Bay K 8644 does not significantly stimulate EDRF release. It has also been reported that Ca^{2+} channel blockers inhibit EDRF release (Singer and Peach, 1982); our results show, however, that both nifedipine and (+)-Bay K 8644 have similar effects on NA- and Ca^{2+} -induced contractions whether the endothelium is present or absent, which suggests that these drugs do not impede either basal or NA-induced EDRF release. In general, our new data obtained with Bay K 8644 enantiomers and $^{45}\text{Ca}^{2+}$ influx provide further evidence against Ca^{2+} channel modulators having any effect on EDRF release, in accordance with others reports (Spedding et al., 1986; Kikkawa et al., 1989). This has implications for the understanding of the action of these drugs on smooth muscle.

The lack of intracellular effects of Bay K 8644 enantiomers and nifedipine is illustrated by the results obtained in Ca^{2+} -free medium. Our results indicate that Bay K 8644 enantiomers and nifedipine do not act intracellularly since, according to previous studies (Godfraind, 1983; Dong and Wadsworth, 1986; Orallo et al., 1991), there is no inhibition of fast transient NA-induced contraction in Ca^{2+} -free medium, whether endothelial cells are present or not.

If the various drug-induced responses observed in this study are expressed as percentage modifications of the response concerned, there were no significant differences between the effects of the dihydropyridines studied on (a) NA-induced contractions and $^{45}\text{Ca}^{2+}$ uptake (probably indicative of effects on ROCs), and

(b) on contractions and $^{45}\text{Ca}^{2+}$ uptake induced by depolarizing medium (indicative of effects on PDCs). Thus our new data concerning the actions of (-)-Bay K 8644 and (+)-Bay K 8644 on rat aorta (similar results with nifedipine have already been reported by Chiu et al. (1986)) are counter to the view that these drugs distinguish ROCs from PDCs, one of the arguments most commonly used in favour of the existence of separate and independent populations of ROCs and PDCs. As has been suggested by Chiu et al. (1986) and Zelis and Moore (1989), this opens the possibility that the two types of channel are interrelated and share similar structural characteristics.

In conclusion, we have evaluated the vasomodulator effects of Bay K 8644 enantiomers and nifedipine on endothelium-rubbed and intact rat aorta rings. Our results show principally that the endothelial system does not modulate the effects of dihydropyridines, and that the effects of these drugs on contractile responses and $^{45}\text{Ca}^{2+}$ uptake elicited by NA and high- K^+ (55 mM) solutions are similar.

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