A comparison of the effects of angiotensin II and Bay K 8644 on responses to noradrenaline mediated via postjunctional α_1 - and α_2 -adrenoceptors in rabbit isolated blood vessels

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1 The effects of angiotensin II (AII) and Bay K 8644 on responses to noradrenaline (NA) mediated via postjunctional α_1 - and/or α_2 -adrenoceptors have been compared in three isolated venous preparations from the rabbit, the lateral saphenous vein, the left renal vein and the ear vein.

2 A similar action of AII and Bay K 8644 was observed only in the lateral saphenous vein; each potentiated responses to NA after isolation of a homogeneous population of postjunctional α_2 -adrenoceptors. However, even in this preparation the mechanism of action for these agents was not identical. The sensitivity of KCl-induced contraction to changes in extracellular calcium ions (reflecting activation of voltage-dependent Ca^{2+} channels) was enhanced by Bay K 8644 but reduced by AII.

3 AII produced a selective facilitation of responses mediated via postjunctional α_2 -adrenoceptors. In the lateral saphenous vein it reduced the effectiveness of prazosin and facilitated responses after isolation of α_2 -adrenoceptors with phenoxybenzamine and rauwolscine. It directly enhanced responses to NA in the ear vein, where only α_2 -adrenoceptors are involved. In contrast, AII did not influence responses mediated via postjunctional α_1 -adrenoceptors in the left renal vein (even after the receptor reserve had been removed with phenoxybenzamine) nor the 'rauwolscine-resistant' component of responses to NA in the saphenous vein.

4 Bay K 8644 enhanced contractile responses to NA mediated both via α_2 -adrenoceptors, in the lateral saphenous vein, and via α_1 -adrenoceptors in the left renal vein. Thus, unlike angiotensin II, no preferential effect was apparent.

5 Bay K 8644 was inactive against responses to NA in the rabbit isolated ear vein. Since the sustained component of responses to NA in this preparation is dependent upon the influx of extracellular Ca^{2+} , these observations suggest that the influx of Ca^{2+} stimulated by NA is mediated via receptor-operated (1,4-dihydropyridine-resistant) Ca^{2+} channels.

Keywords: Noradrenaline; angiotensin II; Bay K 8644; postjunctional α_1 -adrenoceptors; postjunctional α_2 -adrenoceptors

Introduction

Both postjunctional α_1 - and α_2 -adrenoceptor-mediated pressor responses can be clearly demonstrated in whole animals (McGrath *et al.*, 1989). Since their discovery it has been suggested that a number of factors can differentially modulate responses to α -adrenoceptor agonists, such as blood pH and blood gases (McGrath *et al.*, 1982; Grant *et al.*, 1985), calcium channel blockers (CCBs) (van Meel *et al.*, 1981) or angiotensin converting enzyme (ACE) inhibitors (de Jonge *et al.*, 1982; Timmermans *et al.*, 1982). It was initially suggested that α_2 -adrenoceptor-mediated responses were more susceptible to the action of CCBs and ACE inhibitors than were equivalent sized α_1 -adrenoceptor-mediated responses (van Meel *et al.*, 1981; de Jonge *et al.*, 1982; Timmermans *et al.*, 1982), although there is now evidence that both CCBs and ACE inhibitors preferentially inhibit the late phase of the pressor response to a bolus injection of an agonist, irrespective of subtype specificity (O'Brien *et al.*, 1985; McGrath & O'Brien, 1987). The study of modulatory influences on responses mediated via different adrenoceptor subtypes in models such as the pithed rat is limited, however, by a number of factors. These include the biphasic nature of responses to the bolus injection of an agonist, the non-'equilibrium' nature of such responses and by the effects of modulatory drugs, such as ACE inhibitors, on basal blood pressure.

While postjunctional α_1 -adrenoceptors are easily demonstrated in isolated blood vessels, there are relatively few examples of α_2 -adrenoceptor-mediated contractions *in vitro* (e.g. Constantine *et al.*, 1982; Daly *et al.*, 1988a). Therefore, comparative studies of the effects of angiotensin II (AII) and calcium channel facilitators on responses mediated via postjunctional α_1 - and α_2 -adrenoceptors in isolated blood vessels have not previously been attempted. However, in the rabbit lateral saphenous vein, a preparation containing both α -adrenoceptor subtypes (Daly *et al.*, 1988b), AII renders responses to B-HT 920 resistant to the α_1 -adrenoceptor antagonist prazosin, suggesting a selective enhancement of α_2 -adrenoceptor-mediated contractions (Schumman & Lues, 1983). Furthermore, two recent studies suggest a similarity of action for AII and Bay K 8644 in isolated vascular preparations: Sulpizio & Hieble (1987) have shown that Bay K 8644 introduces responses mediated via postjunctional α_2 -adrenoceptors in the canine saphenous artery, while Dunn *et al.* (1989) have observed a similar action for AII in the corresponding arterial preparation from the rabbit. Thus, the facilitatory effect of AII on α_2 -adrenoceptor-mediated responses might be attributable to an action on dihydropyridine-sensitive Ca^{2+} -channels.

In the present study we have examined the influence of AII and Bay K 8644 on responses to noradrenaline (NA) mediated via either α_1 - or α_2 -adrenoceptors in three isolated venous preparations from the rabbit: (1) the rabbit isolated lateral saphenous vein, a preparation containing both α -adrenoceptor subtypes, before and after isolation of a homogeneous population of postjunctional α_2 -adrenoceptors (see Daly *et al.*, 1988c); (2) the rabbit ear vein, which contains almost exclusively α_2 -adrenoceptors (Daly *et al.*, 1988a) and (3) the rabbit renal vein, a preparation containing a homogeneous population of α_1 -adrenoceptors (Daly *et al.*, 1988d).

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Methods

White albino rabbits of either sex weighing 2.3–3.0 kg were killed by stunning followed by exsanguination. Segments of the lateral saphenous vein, left renal vein or ear vein were cleaned of fat and connective tissue *in situ* and placed in an ice-cold Krebs bicarbonate saline. When necessary, preparations were cleaned further with the aid of a dissecting microscope. Segments (3–5 mm in length) were taken from each vein and suspended between two 0.2 mm thick wire supports as described by Hooker *et al.* (1976). The upper support was connected by cotton to a Grass FT03 isometric transducer while the lower support was connected to a glass tissue holder. The preparations were then mounted in 30 ml isolated organ baths under an initial resting tension of 2 g wt. (lateral saphenous vein), 0.5 g wt. (left renal vein), 0.3 g wt. (ear vein) respectively and allowed to relax. Each preparation was bathed in Krebs solution maintained at 37°C and gassed with 95% O₂ plus 5% CO₂.

After a 60 min equilibration period, during which a steady resting tension was achieved, each preparation was exposed to 3 μ M NA and allowed to contract for 10 min. Following complete washout, an additional 1 h equilibration period was allowed before starting the experiment. This procedure was found to minimize changes in the sensitivity of the preparation to further addition of agonists and is similar to the method of Ruffolo *et al.* (1979). Basal tension following the sighting response remained stable for the rest of the experiment at 0.3–0.4 g wt. (lateral saphenous vein), 0.15–0.2 g wt. (left renal vein) and 0.07–0.1 g wt. (ear vein); values previously found to be optimal for maximum responsiveness (Daly *et al.*, 1988d). Isometric contractions were recorded on a Linseis 6052 pen recorder. In all experiments cumulative concentration-response curves (CCRCs) to agonists were constructed by increasing the concentration of agonist in the organ bath by approximately 3 fold increments. In the majority of preparations, contractile responses to NA were not sustained and so addition of the next concentration was made as close to the peak as possible. Following attainment of the maximal control contraction, preparations were washed until complete relaxation was effected and 30–60 min later one of the following experimental protocols was adopted:

- (1) The lateral saphenous vein, left renal vein or ear vein were exposed to either AII or Bay K 8644 15 min before the start of a second CCRC to NA.
- (2) The left renal vein was exposed to phenoxybenzamine (1 nM) for a period of 30 min. The tissues were subsequently washed at least 4 times and 30 min later a CCRC to NA was constructed. After washout and a further 60 min equilibration period another CCRC to NA was constructed. In some experiments AII was added 15 min prior to the final NA CCRC.
- (3) In the lateral saphenous vein, preparations were exposed to various concentrations of prazosin or rauwolscine for a minimum period of 45 min and the NA CCRC repeated. In some tissues, AII (0.05 μ M) or Bay K 8644 (0.03 μ M) was added to the organ bath 15 min prior to the onset of the second CCRC to NA, in the continued presence of antagonist.
- (4) A population of postjunctional α_2 -adrenoceptors was isolated in the lateral saphenous vein as previously described (Daly *et al.*, 1988c). Briefly, tissues were exposed to rauwolscine (1 μ M) 5 min prior to phenoxybenzamine (0.3 μ M) which was left in contact with the tissues for a further 30 min. The tissues were then washed a minimum of seven times over a 45 min period. During the first two washes rauwolscine (1 μ M) was present to maximize protection of α_2 -adrenoceptor. After a further 30 min period a CCRC was obtained to NA. The residual response remaining after the protection protocol is resistant to prazosin but susceptible to rauwolscine (Daly *et al.*, 1988c). After attainment of the maximum response to NA the preparations were washed until complete relaxation was effected. A further period of 60 min was allowed before construction of another CCRC to NA.

In some experiments AII or Bay K 8644 was added 15 min prior to the onset of the final NA CCRC. In others saralasin (0.1 μ M) was added 10 min prior to AII.

- (5) Some experiments were performed on the lateral saphenous vein to examine the effects of AII and Bay K 8644 on the influx of extracellular Ca²⁺ into the cell stimulated either by depolarization or by postjunctional α_2 -adrenoceptors. CCRCs to Ca²⁺ were constructed following exposure to nominally Ca²⁺-free Krebs solution with either 65 mM KCl (exchanged with NaCl on an equimolar basis) or 30 μ M NA (after isolation of postjunctional α_2 -adrenoceptors) added 10 min prior to the start of the Ca²⁺ CCRC. Following attainment of the control maximum response, tissues were washed in nominally Ca²⁺-free Krebs solution (containing 23 μ M EDTA) until complete relaxation was affected and allowed to bathe in this solution. A second Ca²⁺ CCRC (in the presence of high K⁺ or NA) was obtained 45–60 min later in the presence of AII or Bay K 8644.

All responses are expressed as a percentage (mean $\pm$ s.e.mean) of the maximum response of the first concentration-response curve to NA, or to Ca²⁺ re-addition, as appropriate, obtained in each tissue. Differences between means were considered statistically significantly different if $P < 0.05$ for either paired or unpaired observations (Student's *t* test).

The composition of the modified Krebs-Henseleit saline was (mM): NaCl 118.4, KCl 4.7, CaCl₂ 2.5, MgSO₄·7H₂O 1.2, NaHCO₃ 24.9, KH₂PO₄ 1.2 and glucose 11.1. Na₂EDTA 23 μ M was included in all experiments to prevent oxidative degradation of NA. Propranolol 1 μ M and cocaine 10 μ M were included to inhibit β -adrenoceptors and uptake₁, respectively. The following compounds were used: (–)-noradrenaline bitartrate (Sigma), phenoxybenzamine HCl (Smith, Kline & French), rauwolscine HCl (Roth), angiotensin II amide (Ciba), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate; Bayer), (±)-propranolol HCl (Sigma), cocaine HCl (Macarthy's), saralasin ([Sar¹, Ala⁸]-angiotensin II) (Sigma), and prazosin HCl (Pfizer). With the exception of Bay K 8644 and phenoxybenzamine, all drugs were dissolved in distilled water and added to the organ bath in volumes of 0.3 ml or less. Stock solutions of phenoxybenzamine (1 mM) were prepared in 20% absolute ethanol in distilled water and a drop of 1 N HCl was added to remove turbidity. Stock solutions of Bay K 8644 were prepared in 20% absolute ethanol. Further dilutions of both drugs were made in distilled water.

Results

Lateral saphenous vein

Responses to noradrenaline NA (1 nM–10 μ M) produced concentration-dependent contractions of the lateral saphenous vein (Figure 1a). Successive concentration-response curves were associated with an increase in the maximum response (<10% of the 1st maximum), without a change in the pD₂ of the agonist. AII (0.005–0.5 μ M) produced concentration-dependent contractions in the lateral saphenous vein, the maximum response of which ranged between 25–40% of the maximum response to NA. Responses to high concentrations of AII (0.05–0.5 μ M), consisted of an initial rapid response which returned to baseline after 6–8 min. Lower concentrations produced contractions which were slower in onset, longer lasting and returned to baseline after 12–15 min. Baseline tension remained stable after the transient contraction produced by AII, for at least the duration of the experiment.

AII (0.05–0.5 μ M) had no effect on responses to NA (Figure 1a). In marked contrast, 30 nM Bay K 8644, which did not produce a contractile response, increased both the sensitivity

Figure 1 The effects of (a) angiotensin II and (b) Bay K 8644 on noradrenaline (NA)-induced contractile responses of the rabbit isolated saphenous vein. (a) The effect of noradrenaline in the absence (○) and in the presence of 0.05 μM (●) and 0.5 μM (□) angiotensin II. (b) The effect of noradrenaline in the absence (○) and in the presence of 0.03 μM Bay K 8644 (●). All responses have been expressed as a percentage of the maximum response in the first concentration-response curve. Each point represents the mean of $n = 6-10$; s.e.mean shown by vertical bars. * Indicates a significant difference ($P < 0.05$) between responses to noradrenaline in presence and absence of the ancillary agent.

and the maximum response of the preparation to NA (Figure 1b, Table 1). High concentrations of Bay K 8644 ($> 0.3 \mu\text{M}$) induced spontaneous contractions in the lateral saphenous vein.

α -Adrenoceptor antagonist potency In a previous study in the rabbit isolated saphenous vein, it was found that the presence of AII rendered contractile responses to the 'selective' α_2 -adrenoceptor agonist, BHT-920, more resistant to prazosin, suggesting a selective facilitation of α_2 -adrenoceptor-mediated responses (Schümann & Lues, 1983).

In the present study, the inhibition of NA CCRCs produced by $0.01 \mu\text{M}$ prazosin was not significantly affected by $0.05 \mu\text{M}$ AII (Figure 2a), but that produced by $0.1 \mu\text{M}$ and $1 \mu\text{M}$ praz-

Table 1 pD_2 values for noradrenaline (NA) ($\pm$ s.e. of the mean) and E_{max} values (control maximum after correction for time related changes, $E_{\text{max}} = 1$) in the rabbit isolated lateral saphenous vein (LSV), lateral saphenous vein after receptor protection with rauwolscline ($1 \mu\text{M}$) and phenoxybenzamine ($0.3 \mu\text{M}$) (LSVRP), left renal vein (LRV), left renal vein post treatment with phenoxybenzamine (1 nm) (LRVP) and the ear vein (EV)

Preparation	n	pD_2 value		E_{max} AII
		Control	AII	
LSV	10	7.31 ± 0.08	7.40 ± 0.10	1.05 ± 0.05
LSVRP	13	6.83 ± 0.06	$7.18 \pm 0.08^{***}$	$1.18 \pm 0.03^{***}$
LRV	5	6.12 ± 0.19	6.12 ± 0.11	1.02 ± 0.04
LRVP	5	5.76 ± 0.16	5.85 ± 0.17	0.93 ± 0.05
EV	10	7.62 ± 0.07	$7.80 \pm 0.09^*$	1.03 ± 0.07

	n	Control	Bay K 8644	Bay K 8644
LSV	6	7.28 ± 0.13	$7.53 \pm 0.10^*$	$1.22 \pm 0.05^{**}$
LSVRP	6	6.81 ± 0.16	$7.23 \pm 0.20^{***}$	$1.25 \pm 0.05^{***}$
LRV	7	6.06 ± 0.08	$6.24 \pm 0.10^*$	$1.30 \pm 0.03^{***}$
EV	5	7.82 ± 0.09	7.79 ± 0.05	1.09 ± 0.11

Differences between control and treated preparations were considered statistically significant if $P < 0.05$ for either paired or unpaired observations (Student's t test) and are denoted by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

sin, particularly at low NA concentrations, was significantly less in the presence of the peptide (Figures 2b and c). The log concentration-ratio for NA produced by $0.1 \mu\text{M}$ prazosin (measured at the 25% level of the maximum response), was less in the presence of $0.05 \mu\text{M}$ AII but this did not reach significance (Table 2). Similarly, although the pA_2 value for prazosin (8.19 : $8.67-7.72$, 95% confidence limits) was reduced in the presence of $0.05 \mu\text{M}$ AII (7.75 : $7.84-7.26$, 95% confidence limits), this did not reach significance. Furthermore, the slopes of the Schild plot for prazosin in either the presence (0.81 : $0.68-0.93$, 95% confidence limits) or absence (0.63 : $0.51-0.72$, 95% confidence limits) of AII were significantly different from unity, but not from each other.

In marked contrast to the findings in the presence of prazosin, AII ($0.05 \mu\text{M}$) was without effect on the inhibition produced by the selective α_2 -adrenoceptor antagonist rauwolscline: neither the rauwolscline-sensitive nor the rauwolscline-resistant components were affected (Figure 3, Table 1). Bay K 8644 ($0.03 \mu\text{M}$), significantly reduced the inhibitory potency of prazosin against NA contractions (Table 2), such that $1 \mu\text{M}$ prazosin failed to produce any greater inhibition than that observed with $0.1 \mu\text{M}$ prazosin (Figures 4a and b). Thus, in the presence of Bay K 8644 it was no longer possible to determine a pA_2 value for prazosin against NA.

After α_2 -adrenoceptor isolation After isolation of postjunctional α_2 -adrenoceptors, both AII and Bay K 8644 markedly enhanced responses to NA (Figure 5, Table 1). This was associated with an increase in both the sensitivity and

Table 2 Log agonist concentration-ratio values for noradrenaline (NA) measured at the 25%, 50% and 75% level of the maximum response for NA in the presence of prazosin ($0.1 \mu\text{M}$) or rauwolscline ($0.05 \mu\text{M}$) and the effects of angiotensin II (AII, $0.05 \mu\text{M}$) and Bay K 8644 ($0.03 \mu\text{M}$) thereon

Antagonist	n	25%	50%	75%
Prazosin ($0.1 \mu\text{M}$)	12	0.81 ± 0.07	0.69 ± 0.07	0.64 ± 0.10
Prazosin ($0.1 \mu\text{M}$) with AII ($0.05 \mu\text{M}$)	7	0.60 ± 0.09	0.57 ± 0.08	0.59 ± 0.09
Prazosin ($0.1 \mu\text{M}$) with Bay K 8644 ($0.03 \mu\text{M}$)	5	$0.49 \pm 0.08^*$	$0.35 \pm 0.09^*$	$0.33 \pm 0.08^*$
Rauwolscline ($0.5 \mu\text{M}$)	5	1.47 ± 0.14	1.70 ± 0.12	1.85 ± 0.09
Rauwolscline ($0.5 \mu\text{M}$) with AII ($0.05 \mu\text{M}$)	5	1.38 ± 0.18	1.73 ± 0.08	1.86 ± 0.07

* Denotes a significant difference in the value for prazosin ($0.1 \mu\text{M}$) calculated at the 25%, 50% and 75% levels of the maximum response to NA in the presence of Bay K 8644 compared to control. $P < 0.05$ Student's t test.

Figure 2 The effects (a) $0.01 \mu\text{M}$, (b) $0.1 \mu\text{M}$ and (c) $1 \mu\text{M}$ prazosin against noradrenaline (NA)-induced contractions of the rabbit isolated saphenous vein in the absence (●) and in the presence of (□) $0.05 \mu\text{M}$ angiotensin II. The control concentration-response curve to NA prior to exposure to prazosin and angiotensin II is represented by (○). Each point represents the mean of 5–6 observations; vertical bars show s.e.mean. * Indicates a significance difference ($P < 0.05$) between responses to noradrenaline remaining after prazosin in presence and absence of angiotensin II.

maximum response of the preparation to NA. It is interesting to note that this facilitatory action of AII was observed with both $0.05 \mu\text{M}$ and 5 nM AII, the latter concentration producing a small contraction in only 2 of 6 preparations. The potentiation of α_2 -adrenoceptor-mediated responses produced by AII was not observed if the angiotensin receptor antagonist, saralasin ($0.1 \mu\text{M}$) was included.

Sensitivity to Ca^{2+} ; comparison of direct depolarization and α_2 -adrenoceptor activation The superficial similarity of the

Figure 3 The effects of (a) $0.05 \mu\text{M}$ and (b) $2.5 \mu\text{M}$ rauwolscine against noradrenaline (NA)-induced contractions of the rabbit isolated saphenous vein in the absence (●) and presence (□) of $0.05 \mu\text{M}$ angiotensin II. The control concentration-response curve to NA prior to exposure to rauwolscine and angiotensin II is represented by (○). Each point represents the mean of 5–6 observations; vertical bars show s.e.mean.

action of AII and Bay K 8644 in relation to the α_2 -adrenoceptor function in the lateral saphenous vein (both agents enhanced responses) prompted a study of their effects on the influx of extracellular Ca^{2+} associated with either direct depolarization (KCl) or activation of postjunctional α_2 -adrenoceptors by NA.

After prolonged exposure to 'nominally Ca^{2+} -free' Krebs-Henseleit saline, no response was observed to the addition of either $0.03 \mu\text{M}$ Bay K 8644 or $0.05 \mu\text{M}$ AII, nor to the subsequent addition of extracellular Ca^{2+} ions (0.01 mM – 2.5 mM). In marked contrast, the exchange of 'nominally Ca^{2+} -free' Krebs-Henseleit for 65 mM KCl 'nominally Ca^{2+} -free' Krebs-Henseleit solution (with equimolar replacement of NaCl) was associated with a small contraction in most preparations (equivalent to 5–10% of the maximum response observed upon readdition of 2.5 mM Ca^{2+}). Subsequent addition of Ca^{2+} ions (0.01 mM – 2.5 mM) was associated with concentration-related contractions (Figure 6a). Consecutive Ca^{2+} CCRCs in the presence of 65 mM KCl were superimposable (Figure 6a).

The addition of AII ($0.05 \mu\text{M}$) prior to the second Ca^{2+} CCRC was associated with a significant rightward displacement of the Ca^{2+} CCRC (pD_2 for Ca^{2+} = 3.49 ± 0.07 and 3.29 ± 0.07 , $n = 9$; $P < 0.05$, Figure 6c) without altering the maximum response. In contrast, Bay K 8644 ($0.03 \mu\text{M}$) failed to alter the sensitivity of the depolarized preparation to Ca^{2+} (pD_2 = 3.47 ± 0.06 and 3.46 ± 0.06 , $n = 8$) but produced a small but significant increase in the contractile response to Ca^{2+} readdition (Figure 6c).

After isolation of postjunctional α_2 -adrenoceptors and exposure to 'nominally Ca^{2+} -free Krebs solution', NA ($30 \mu\text{M}$) produced a small transient contraction ($7.7 \pm 1.9\%$ of the maximum response to readdition of Ca^{2+} ions, $n = 9$), which

Figure 4 The effects of 0.01 μM (●), 0.1 μM (□) and 1 μM (■) prazosin against noradrenaline (NA)-induced contractions of the rabbit isolated saphenous vein in (a) the absence and (b) the presence of 0.03 μM Bay K 8644. The control concentration-response curve to NA prior to exposure to prazosin is represented by (○), and all responses have been expressed as a percentage of the first concentration-response curve in the absence of both prazosin and Bay K 8644. Each point represents the mean of 5–6 observations; vertical bars show s.e.mean.

returned to baseline within 10 min. Consecutive CCRCs to the re-addition of Ca^{2+} under these conditions was associated with a small reduction in the maximum attainable response, but no change in sensitivity (Figure 6b). AII (0.05 μM) produced a small increase in the maximum response to the re-addition of Ca^{2+} , although this was not associated with a change in sensitivity of the preparation to Ca^{2+} (Figure 6d). In contrast Bay K 8644 increased both the sensitivity and the maximum response to the re-addition of Ca^{2+} , upon stimulation of postjunctional α_2 -adrenoceptors with NA (Figure 6d).

Ear vein

NA produced concentration-dependent contractions in the isolated ear vein, a preparation containing predominantly postjunctional α_2 -adrenoceptors (Daly *et al.*, 1988a). Consecutive CCRCs to NA were not reproducible with time: there was an approximately 25% increase in the maximum response, but no change in sensitivity (pD_2 for NA, 7.82 ± 0.08 and 7.83 ± 0.08 for curves 1 and 2 respectively, $n = 11$). AII 0.05 μM , produced a transient contraction equivalent to $46.3 \pm 8.3\%$ of the NA maximum response ($n = 11$) and, after correcting for time-related changes, produced a small, but significant, increase in the sensitivity of the preparation to NA (pD_2 for NA 7.62 ± 0.07 and 7.84 ± 0.08 in the absence and presence of 0.05 μM AII, $P < 0.05$ for paired Student's *t* test, $n = 10$). In contrast, the addition of Bay K 8644 (0.03 μM) was not associated with a contractile response and failed to alter responses to NA.

Figure 5 The effects of angiotensin II and Bay K 8644 on noradrenaline (NA)-induced contractile responses of the rabbit isolated saphenous vein after isolation of postjunctional α_2 -adrenoceptors. (a) Noradrenaline-induced contractions in the (●) absence and in the (□) presence of 5 nM angiotensin II. (b) Noradrenaline-induced contraction in the (●) absence and in the (□) presence of 0.05 μM angiotensin II. (c) Noradrenaline-induced contractions in the (●) absence and in the (■) presence of 0.03 μM Bay K 8644. All responses have been expressed as a percentage of the maximum response in the first concentration-response curve (○) prior to the receptor protection procedure. Each point represents the mean of $n = 6$ –13; vertical bars show s.e.mean. * Indicates a significant difference ($P < 0.05$) between responses to noradrenaline in presence and absence of the ancillary agent.

Left renal vein

The rabbit isolated left renal vein contains a homogeneous population of postjunctional α_1 -adrenoceptors (Daly *et al.*, 1988d). Consecutive CCRCs to NA in this preparation were reproducible. AII (0.05 μM) produced a transient contraction in the left renal vein, which was equivalent to $31.9 \pm 8.7\%$ of the NA maximum response ($n = 11$). This concentration of AII was without effect on responses to NA (Figure 7a). In

Figure 6 The effect of angiotensin II and Bay K 8644 on Ca^{2+} concentration-response curves in the rabbit isolated saphenous vein following depolarization or α_2 -adrenoceptor stimulation. (a,b) The reproducibility of Ca^{2+} concentration-response curves following exposure to nominally Ca^{2+} -free Krebs-Henseleit saline and then (a) 65 mM KCl Krebs-Henseleit saline or (b) α_2 -adrenoceptor stimulation with 30 μM noradrenaline (NA): (○) 1st concentration-response curve and (●) 2nd concentration-response curve. (c,d) The effects of (●) 0.05 μM angiotensin II and (□) 0.03 μM Bay K 8644 on contractile responses elicited by the re-addition of Ca^{2+} , following exposure to 'nominally Ca^{2+} -free' Krebs-Henseleit saline, in the presence of (c) 65 mM KCl Krebs-Henseleit saline and (d) 30 μM NA after isolation of postjunctional α_2 -adrenoceptors. Results are expressed as a % of the maximum response in the control curve for Ca^{2+} readdition. Each point represents the mean of $n = 6-8$; vertical bars show s.e.mean. * Indicates a significant difference ($P < 0.05$) between responses to noradrenaline in presence and absence of the ancillary agent.

Figure 7 The effects of angiotensin II and Bay K 8644 on noradrenaline (NA)-induced contractions of the rabbit isolated left renal vein. (a) Responses to noradrenaline in the (●) presence and (○) absence of 0.05 μM angiotensin II. (b) Responses to noradrenaline in the (●) presence and (○) absence of 0.3 μM Bay K 8644. (c) Responses to noradrenaline, following 30 min exposure to 1 nM phenoxybenzamine and subsequent washout, in the (●) presence and (□) absence of 0.05 μM angiotensin II. Responses to noradrenaline prior to exposure to phenoxybenzamine are represented by (○). Each point represents the mean of $n = 5-7$; vertical bars show s.e.mean. * Indicates a significant difference ($P < 0.05$) between responses to noradrenaline in presence and absence of the ancillary agent.

contrast, Bay K 8644 (0.03 μM), which again had no effect on resting baseline tension, increased the sensitivity and the maximum response of the preparation to NA (Figure 7b).

It has previously been shown that reducing the receptor reserve in the rabbit femoral artery increased the facilitatory effect of AII on responses to NA mediated via α_1 -adrenoceptors (Purdy & Weber, 1988). Therefore, the left renal vein was exposed to phenoxybenzamine (1 nM) for 30 min to reduce the receptor reserve. This produced a reduction of $42.0 \pm 9.3\%$ (range 17–75%, $n = 6$) in the maximum response

to NA (Figure 7c), although there was no change in the sensitivity of the preparation to NA (pD_2 for NA, 5.91 ± 0.04 and 5.92 ± 0.11 , before and after treatment with phenoxybenzamine respectively, $n = 6$). In two further preparations, phenoxybenzamine abolished all responses and in another it failed to reduce the maximum response. These results have been excluded from the analysis. Even under these conditions, AII ($0.05 \mu M$) had no effect on the residual response remaining after phenoxybenzamine (Figure 7c).

Discussion

Comparison of the actions of angiotensin II and Bay K 8644

ACE inhibitors and Ca^{2+} channel blockers (CCB) have qualitatively similar effects on α -adrenoceptor-mediated pressor responses in the pithed rat (van Meel *et al.*, 1981; De Jonge *et al.*, 1981; 1982; O'Brien *et al.*, 1985). In complementary experiments on isolated blood vessels, the Ca^{2+} channel facilitator Bay K 8644 and AII have been shown to 'unmask' responses mediated via postjunctional α_2 -adrenoceptors in the canine and rabbit saphenous arteries, respectively; effects produced in the absence of a sustained elevation of vascular tone (Sulpizio & Hieble, 1987; Dunn *et al.*, 1989). Furthermore, in the rabbit isolated saphenous vein the presence of both AII and Bay K 8644 was associated with a reduction in the inhibitory potency of the α_1 -adrenoceptor antagonist, prazosin (present study). Taken together, these observations suggest that both agents may influence α -adrenoceptor-mediated responses via a common mechanism involving dihydropyridine-sensitive Ca^{2+} channels.

However, a comparison of the effects of AII and Bay K 8644 in three isolated venous preparations from the rabbit revealed several differences. With the exception of the ear vein, Bay K 8644 produced a small but significant enhancement of responses to NA in all preparations tested irrespective of the receptor involved. On the other hand, AII potentiated only responses mediated by α_2 -adrenoceptors responses in the rabbit isolated ear vein and lateral saphenous vein (the latter only manifest after irreversible inactivation of post-junctional α_1 -adrenoceptors).

Although both agents produce qualitatively similar effects against α_2 -adrenoceptor function in the rabbit isolated saphenous vein, the essential difference in the mode of action of these two agents is underlined by studies on voltage-operated Ca^{2+} channels; the presumed site of action of Bay K 8644 (Schramm *et al.* 1983; Su *et al.*, 1984). Bay K 8644 enhanced Ca^{2+} -induced contractions in the presence of a depolarizing concentration of KCl, without altering the sensitivity of the voltage-operated Ca^{2+} channel. The unexpectedly small effect may be a consequence of the degree of depolarization produced by 65 mM KCl, i.e. the response cannot be further facilitated. In marked contrast, AII inhibited the Ca^{2+} -induced contractions mediated via voltage-operated channels, an effect associated with a small reduction in channel sensitivity to Ca^{2+} . The basis for this effect is not clear, but the possibility that AII produces a vasodilator prostaglandin is worthy of investigation.

Since α_2 -adrenoceptor contractions in the rabbit isolated saphenous vein are entirely dependent upon the entry of extracellular Ca^{2+} (Daly *et al.*, 1990), and only part of this is via dihydropyridine-sensitive Ca^{2+} channels (Dunn *et al.*, 1991b), it seems likely that the similar facilitatory effects of Bay K 8644 and AII against α_2 -adrenoceptor-mediated contractions arise from discrete effects on dihydropyridine-sensitive and dihydropyridine-resistant Ca^{2+} channels, respectively. The precise relationship between α -adrenoceptor subtype, voltage-operated Ca^{2+} channels and

dihydropyridine-sensitive Ca^{2+} channels in the rabbit isolated saphenous vein awaits detailed electrophysiological studies.

Effects of angiotensin II on responses mediated via postjunctional α_1 - and α_2 -adrenoceptors

A number of observations in the present study support a preferential interaction between AII and responses mediated via postjunctional α_2 -adrenoceptors rather than α_1 -adrenoceptors. First, AII produced a modest reduction in the potency of prazosin against responses to NA in the lateral saphenous vein. These results are essentially similar to those obtained by Schümann & Lues (1983) in the same preparation, who demonstrated that responses to BHT-920, which under normal circumstances were markedly inhibited by prazosin, became much more resistant to the α_1 -adrenoceptor antagonist in the presence of AII. Secondly, AII produced a small but significant enhancement of responses to NA in the rabbit isolated ear vein, a preparation containing an almost homogeneous population of postjunctional α_2 -adrenoceptors (Daly *et al.*, 1988). Thirdly, AII failed to modify the antagonism of NA contractions produced by rauwolscine in the rabbit isolated saphenous vein; the 'rauwolscine-resistant' component (previously shown to be prazosin-sensitive; Daly *et al.*, 1988b) is similar in the absence and presence of AII (Figure 3b). Finally, in the left renal vein, a preparation containing a homogeneous population of postjunctional α_1 -adrenoceptors (Daly *et al.*, 1988d), AII was without effect on responses to NA.

It has been suggested that the presence of a large 'receptor reserve' for α -adrenoceptors can limit functional antagonism produced by either Ca^{2+} channel blockers (Nichols & Ruffolo, 1988) or a reduction in temperature (Flavahan & Vanhoutte, 1986). Since the corollary of this is that scope for facilitation is similarly reduced in a system with a large receptor reserve, we also investigated the effect of AII on α -adrenoceptor function in the renal vein after exposure to phenoxybenzamine. Phenoxybenzamine produced a reduction in the maximum response to NA, without altering the sensitivity of the preparation, but this procedure failed to uncover a facilitatory effect of AII.

These observations fail to generalize those of Purdy & Weber (1988) who were able to demonstrate a facilitation of ' α_1 -adrenoceptor'-mediated responses in rabbit isolated femoral artery, this effect being particularly evident following exposure to the irreversible antagonist, benextramine. However, two observations suggest that postjunctional α_2 -adrenoceptors may be present in the rabbit isolated femoral artery and, as such, provide an alternative basis for the observed potentiation. First, AII uncovers previously unseen responses to the selective α_2 -adrenoceptor agonist UK-14304 in the rabbit femoral artery (authors, unpublished observations). Secondly, Purdy & Weber (1988) noted that following irreversible inactivation of α -adrenoceptors with benextramine and subsequent exposure to AII, responses of the rabbit isolated femoral artery to NA exhibited slightly greater resistance to prazosin. With respect to this latter observation, there are clear parallels with findings in the rabbit isolated saphenous vein (present study) and the rabbit isolated distal saphenous artery (Dunn *et al.*, 1991a).

Further support for a preferential interaction between AII and postjunctional α_2 -adrenoceptors is provided by several reports that predate the subclassification of postjunctional α -adrenoceptors. For example, AII was found to potentiate responses to NA in a number of isolated vascular preparations such as perfused mesenteric arteries from a number of species (Malik & Nasjletti, 1976; Chiba & Tsukada, 1986; Panisset & Bourdois, 1968), human digital arteries (Moulds & Worland, 1980) and in canine hindpaw (Zimmerman & Kraft, 1979). It is noteworthy that a re-examination of the α -adrenoceptor population in many of these preparations has revealed the presence of the α_2 -subtype as in human digital

arteries (Glusa & Mardwardt, 1983; Stevens & Moulds, 1985); feline mesenteric vascular beds (Lippton *et al.*, 1987) and canine saphenous artery (Sulpizio and Hieble, 1987).

It should be noted, however, that while the above evidence favours a preferential interaction between α_2 -adrenoceptors and AII, it does preclude the possibility that AII can also facilitate α_1 -adrenoceptor responses. This is underlined by the report of Day & Moore (1976) which showed a non-specific facilitatory action for AII on responses to various contractile agents, including NA, in rabbit isolated aorta. Similarly, Chiba & Tsukada (1986) also observed an increase in response of the canine mesenteric artery to KCl, as well as NA, in the presence of AII. Since ouabain produced similar effects, these authors concluded that AII modulated responses to vasoactive agents by producing a small depolarization of the cell membrane, thereby reducing the threshold for contraction. However, a non-specific sensitization of vascular smooth muscle is unlikely to account for the effects of AII in the present study since, when the full receptor complement was present in the saphenous vein, responses to bradykinin (unpublished observations) and NA were unaffected by AII. It seems more likely that AII is producing a particular pattern of effects on the signalling process which selectively synergizes with the coupling involved with many α_2 -adrenoceptor-activated processes. There is no reason to suppose that this is either ubiquitous, unique to AII or selective only to α_2 -adrenoceptors but it does seem to be widespread in blood vessels.

Finally, the facilitatory effect of AII on α_2 -adrenoceptor responses in the rabbit isolated saphenous vein was blocked by the AII receptor antagonist, saralasin (Zimmerman & Kraft, 1979), indicating an effect mediated via specific AII receptors. Interestingly, despite the fact that saralasin (0.01 μ M) virtually abolished contractile responses to AII, a 10 fold higher concentration of the antagonist was required to reverse completely the facilitatory action of AII. These results represent a dissociation between the concentrations of AII required for a facilitatory action on responses to NA and a direct solo action on vascular smooth muscle, such that the former can occur with subcontractile concentrations of AII. In accord with this idea, 5 nM AII also produced a consistent facilitation of postjunctional α_2 -adrenoceptor-mediated responses, yet it produced a very small contraction in only 2 of 6 preparations.

Effects of Bay K 8644 on responses mediated via postjunctional α_1 - and α_2 -adrenoceptors

The sustained component of responses to NA in rabbit isolated lateral saphenous vein and left renal vein has been shown to be largely dependent on the influx of extracellular Ca^{2+} (Daly *et al.*, 1990). No evidence was obtained in these preparations to suggest that Bay K 8644 differentially influenced responses mediated via either postjunctional α_1 - or α_2 -adrenoceptors. Both could be enhanced. For example, Bay K 8644 produced an enhancement of responses mediated via α_2 -adrenoceptors in the lateral saphenous vein. This was suggested by the reduction in potency of prazosin against responses to NA in the presence of Bay K 8644 and was demonstrated directly after isolation of a homogeneous popu-

lation of postjunctional α_2 -adrenoceptors. Bay K 8644 also potentiated responses to NA mediated via postjunctional α_1 -adrenoceptors in the left renal vein.

In marked contrast, however, Bay K 8644 was without effect on responses to NA mediated via postjunctional α_2 -adrenoceptors in the isolated ear vein. Since the sustained component of responses to NA in this preparation are also largely dependent upon extracellular Ca^{2+} (Daly *et al.*, 1990), responses to NA probably reflect the entry of Ca^{2+} via 1,4-dihydropyridine-resistant, receptor-operated channels. In agreement with this hypothesis, nifedipine is ineffective against responses to NA in the ear vein (Dunn *et al.*, 1991b).

The few other comparative studies which have been attempted *in vitro* further outline the dangers inherent in generalizing the susceptibility of responses mediated via a particular receptor to the blocking action of agents which interfere with one aspect of stimulus-response coupling. For example, while Bay K 8644 can enhance postjunctional α_2 -adrenoceptor-mediated responses in a number of vascular preparations such as the rat saphenous vein (Cheung, 1985), feline mesenteric bed (Lippton *et al.*, 1987) or canine saphenous artery (Sulpizio & Hieble, 1987), responses to NA, in the presence of prazosin (α_2 -adrenoceptor-mediated), are unaffected by Bay K 8644 in the human hand vein (Arner *et al.*, 1988). In addition, postjunctional α_1 -adrenoceptor-mediated responses are facilitated by Bay K 8644 and reduced by nimodipine in human hand veins (Arner *et al.*, 1988), but in contrast are resistant to the action of Bay K 8644 in the canine saphenous vein (Eskinder & Gross, 1987).

Therefore a sufficient number of exceptions can be demonstrated in isolated vascular preparations, to prevent the direct association between α -adrenoceptor subtype and susceptibility to 1,4-dihydropyridine derivatives, which has been postulated *in vivo*. The differential effect of CCBs in whole animals is more likely to reflect a limitation of measuring only the change in the peak response to the bolus injection of an agonist. For example, CCBs have no differential effect on responses mediated via either postjunctional α_1 - or α_2 -adrenoceptors, if the area under the curve is used as a measure of the whole response to a given agonist (McGrath & O'Brien, 1987). Likewise, these agents reduce the size of the responses to infusions, which give a more sustained type response, of agonists selective for either subtype (McGrath & O'Brien, 1987; Lefevre-Borg *et al.*, 1988).

In summary, in three isolated venous preparations from the rabbit, a comparison of the actions of AII and Bay K 8644 revealed marked differences in their effects on responses to NA. AII appears to produce a preferential facilitation of responses mediated via postjunctional α_2 -adrenoceptors. In contrast, Bay K 8644 facilitated responses to NA in two of the three preparations (with the exception of the ear vein) irrespective of the particular α -adrenoceptor involved in mediating the contraction.

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