Angiotensin II Amplification of α-Adrenergic Vasoconstriction: Role of Receptor Reserve

Ralph E. Purdy and Michael A. Weber

Angiotensin II (Ang II) is known to enhance the vasoconstrictor response to norepinephrine (NE). In the present study, this interaction was investigated using isolated rabbit femoral artery rings mounted in tissue baths for the measurement of isometric contraction. Exposure to \(3 \times 10^{-10} \text{ M} \) Ang II caused a contraction that was less than 5% of the maximal response to NE. In the presence of Ang II, the NE dose-response curve shifted to the left twofold and the maximal response was not changed. The calcium channel antagonist nifedipine, \(1 \times 10^{-7} \text{ M} \), caused a modest inhibition of the response to NE in either the presence or absence of Ang II. In contrast, nifedipine abolished the leftward shift of the NE dose-response curve caused by Ang II. Femoral arteries were pretreated with benextramine to cause partial α-adrenoceptor inactivation. The maximal contractile response to NE in these tissues was between 20% and 40% of that in control vessels, indicating that α-adrenoceptor reserve had been eliminated. In benextramine-pretreated vessels, the presence of \(3 \times 10^{-10} \text{ M} \) Ang II caused a modest leftward shift of the NE dose-response curve but increased the maximal responses to all NE concentrations by 200% to 800%. Nifedipine caused a modest inhibition of the response to NE in the absence of Ang II. In contrast, the enhanced response to NE in the presence of Ang II was nearly abolished. These results support our conclusions that 1) Ang II enhances the vasoconstrictor response to α-adrenergic stimulation, 2) the magnitude of enhancement is greater under conditions of reduced α-receptor reserve, and 3) calcium channel activation plays a major role in the amplified response. (Circulation Research 1988;63:748–757)

Norepinephrine (NE) and angiotensin II (Ang II) are powerful endogenous vasoconstrictor substances, each playing an important role in the regulation of cardiovascular function. In addition, there is a large body of evidence that Ang II has a marked effect on the response of the cardiovascular system to sympathetic activation. For example, Ang II enhances the response of isolated blood vessels and other organs to electrical stimulation of sympathetic nerves. This is thought to be mediated by activation of Ang II receptors on the nerve terminals, resulting in an increased release of adrenergic neurotransmitter. Ang II also increases the release of catecholamines from the adrenal medulla.

In addition to its effects on sympatheoadrenal function, Ang II also sensitizes the cardiovascular system to the application of exogenous catecholamines. This has been observed in vivo as an enhancement of the pressor response to injected NE, and in vitro as an enhanced contractile response to NE in blood vessel rings and strips. Ang II has been shown to block the neuronal uptake of catecholamines. However, given the high concentrations of Ang II required, several authors have questioned the contribution of this mechanism to the Ang II-induced enhancement of response to exogenous catecholamines. Ang II appears to act directly on vascular smooth muscle to facilitate α-adrenergic activation.

In the authors' laboratories, the interest in an interaction between Ang II and NE began with a study in the conscious rabbit showing that an infusion of a subpressor dose of Ang II resulted in an enhanced pressor response to NE. The results of that study suggested that the enhancement was not due to alterations in endogenous sympathetic mechanisms or the uptake of NE. In addition, effects of Ang II on sodium retention were ruled out. Thus, it seemed likely that the enhancing effect of the subpressor infusion of Ang II occurred at the level of vascular smooth muscle. Other authors have also identified vascular smooth muscle as the likely site...
for the enhancing effect of Ang II on the pressor response to NE.\(^7\)

To study the interaction between Ang II and NE in greater detail, the experiments of the present study used isolated segments of rabbit femoral artery. NE dose-response curves were obtained in the presence and absence of a contractile threshold dose of Ang II, and experimental manipulations were used to explore the role of \(\alpha\)-adrenergic receptor reserve and calcium channels in the interaction. The results support our conclusions that 1) Ang II enhances the vasoconstrictor response to \(\alpha\)-adrenergic stimulation, 2) the magnitude of enhancement is greater under conditions of reduced \(\alpha\)-receptor reserve, and 3) calcium channel activation plays a major role in the amplified response.

### Materials and Methods

Male New Zealand White rabbits weighing 2.0–2.5 kg were lightly anesthetized (pentobarbital \(\text{Na}^+\); 35 mg/kg i.p.), decapitated, and the femoral arteries were removed, cleaned and cut into 3-mm long rings. In preliminary studies it was found that removal of the endothelium had no effect on the Ang II–induced amplification of the femoral artery contractile responses to NE. Thus, no effort was made to either preserve or eliminate the endothelium in the present study. These blood vessel rings were then mounted for the measurement of isometric tension development in tissue baths containing 30 ml of 95% \(\text{O}_2\)-5% \(\text{CO}_2\) gassed Krebs-bicarbonate solution at 37°C. The composition of the Krebs solution in millimoles per liter was \(\text{NaCl} 119.2\), \(\text{KCl} 4.9\), \(\text{CaCl}_2 1.3\), \(\text{MgSO}_4 1.2\), \(\text{NaHCO}_3 25\), glucose 11.1, ascorbic acid 0.114, and disodium EDTA 0.03.

Femoral arteries were equilibrated under a final resting force of 1.5 g for 30 minutes. Subsequently, all tissues were exposed to 68 mM \(\text{K}^+\) by adding stock \(\text{K}^+\) solution to the tissue bath. When the tissues had reached steady-state contraction, the tissue baths were drained and refilled with fresh Krebs’ solution twice and the tissues were allowed to relax back to baseline. This exposure to 68 mM \(\text{K}^+\) was repeated at least twice with 20–30-minute intervals between. In general, each tissue gave uniform responses to the last two exposures to high \(\text{K}^+\). The average response, in grams, of femoral artery rings to the last exposure to high \(\text{K}^+\) was 7.67 ± 0.13 SEM (\(n = 99\)). All subsequent contractile responses in each vessel were expressed as a percentage of the last contractile response to 68 mM \(\text{K}^+\) in that vessel. Resting force was readjusted as necessary after each tissue wash. In some experiments, tissues were exposed to \(5 \times 10^{-7}\) M benextramine for 30 minutes. The baths were then drained and refilled with fresh Krebs solution four times over 10 minutes. Benextramine is an irreversible \(\alpha\)-adrenergic antagonist that is nonselective between the \(\alpha_1\) and \(\alpha_2\) subtypes.\(^{17}\)

After equilibration and, in some cases, benextramine pretreatment, \(3 \times 10^{-10}\) M Ang II was added to some of the tissue baths, causing a contraction equal to or less than 5% of the maximal response to NE. When this contraction had reached steady state, NE dose-response curves were obtained by cumulative addition to the tissue bath in \(\frac{1}{2}\) log dose increments. Whenever required to satisfy the criteria for valid pharmacological receptor analysis,\(^{19}\) NE dose-response curves were obtained in the presence of \(3 \times 10^{-5}\) M cocaine and deoxy-corticosterone acetate and \(1 \times 10^{-6}\) M propranolol. These agents were added to the tissue bath 30 minutes before exposure to NE to block neuronal\(^{20,21}\) and extra neuronal\(^{22,23}\) uptake of catecholamines and \(\beta\)-adrenergic receptors,\(^{24}\) respectively. These blocking agents had no effect on the NE dose-response curve in benextramine-pretreated blood vessel rings. This may indicate that the neuronal and extraneuronal uptake processes were already saturated by the high NE concentrations required to stimulate these tissues. In some experiments, either prazosin, rauwolscine, or nifedipine was added to the tissue bath 30 minutes before obtaining the NE dose-response curve. Prazosin and rauwolscine antagonist dissociation constants (\(K_b\)) were determined according to the following equation: \(K_b = B/(\text{dr} - 1)\), where B is the antagonist concentration and \(\text{dr}\) is the dose-ratio or magnitude of rightward shift of the agonist dose-response curve caused by the antagonist.\(^{19}\)

Nifedipine, \(1 \times 10^{-7}\) M, was chosen for use in the present study because of evidence showing that its actions at this concentration may be restricted to antagonism of the calcium channel. Nifedipine, \(1 \times 10^{-7}\) M, had little or no effect on radioligand binding to \(\alpha\)-receptors in rat brain membranes,\(^{25}\) and its blockade of the contractile response of rabbit aorta to NE was identical to that obtained with \(1 \times 10^{-7}\) M nifedipine.\(^{26}\) These observations imply a lack of effect of nifedipine on \(\alpha\)-adrenoceptors.

The results of the present study show that \(1 \times 10^{-7}\) M nifedipine had no effect on the contractile response of femoral artery rings to \(3 \times 10^{-10}\) M Ang II (the concentration of Ang II used in the present study). In addition, we have observed that nifedipine has no effect on the Ang II dose-response curve between threshold and 20% of maximal response. Nifedipine modestly reduces the maximal response to Ang II (M.A. Weber and R.E. Purdy, unpublished observations). These observations suggest that nifedipine has no effect on Ang II receptors.

The relation between contractile response and receptor occupancy was determined by the graphical analysis of Furchgott.\(^{27}\) Based on mass law assumptions, Furchgott derived the following equation, which describes the relation between efficacious concentrations of agonist in control (A) and benextramine-treated (A') tissues:

\[
\frac{1}{[A]} = \frac{1}{q[A']} + \frac{(1-q)qK_A}{(1-q)K_A}
\]

where \(q\) is the fraction of active receptors remaining after benextramine treatment. If \(1/[A]\) is plotted against
of nifedipine, the NE curves in the presence and absence of Ang II were superimposed. Several authors have suggested that a large receptor reserve can buffer or attenuate the inhibition of contractile response caused by calcium channel antagonists,29 organic nitrates,30 or cooling.31 This raised the possibility that if the femoral artery possesses a large \( \alpha \)-adrenoceptor reserve, reduction of this reserve may unmask a larger enhancing effect of Ang II. To test this, femoral artery rings were exposed to benextramine prior to obtaining NE dose-response curves. This treatment reduced the response to \( 1 \times 10^{-4} \) M NE to 20–40% of the maximal contractile response obtained in untreated femoral artery rings. The effect of Ang II on the NE dose-response curve in benextramine-pretreated artery rings is shown in Figure 3. Ang II enhanced the response to NE throughout the NE dose-response curve.

Since \( 3 \times 10^{-10} \) M Ang II elicits a small contraction in the femoral artery, this contraction itself could account, at least in part, for the enhancing effect of Ang II on the NE dose-response curve. To
test this, NE dose-response curves were obtained in the presence and absence of either the 3 × 10⁻¹⁰ M Ang II or a concentration of NE (1 × 10⁻⁹ M) sufficient to cause a contraction equal to that elicited by Ang II. As seen in Figure 4, prior contraction with NE caused a small, nonsignificant enhancement of the NE dose-response curve. In contrast, Ang II caused a marked significant enhancement of the response to NE throughout the dose-response curve.

The effects of nifedipine on the Ang II NE interaction were assessed in benextramine-pretreated femoral arteries and the results are shown in Figure 5. Nifedipine caused a modest, noncompetitive inhibition of the contractile response to NE in the absence of Ang II. In contrast, the enhanced response to NE in the presence of Ang II was nearly abolished. The effect of adding Ang II at the end of the NE dose response curve is also shown in Figure 5. Tissues exposed to Ang II at the end of the experiment contracted to the same level as those exposed to Ang II prior to the NE dose-response curve. In addition, the contractile effect of Ang II added at the end of the NE dose-response curve was markedly inhibited in the presence of nifedipine.

It is possible that either Ang II exposure or benextramine pretreatment could have altered the nature of the α-adrenoceptor on which NE acts and this, in turn, could account for the enhanced response to NE. As a first test of this, dose-response curves to NE were obtained in the presence and absence of either 3 × 10⁻¹⁰ M angiotensin II (AlI) or a concentration NE (1 × 10⁻⁹ M) causing a contraction equal to that of angiotensin II. Vertical bars represent SEM. Contraction is expressed as a percentage of the contractile response to 68 mM K⁺ (% of STD). n=12.
FIGURE 6. Dose-response curves for the contractile effect of (NE) in the femoral artery, in the absence and presence of either 1x10^-8 (A and B) or 2x10^-9 M (C) prazosin. Vertical bars represent SEM. Angiotensin was absent in the experiments in panel A and present in those in panels B and C. The femoral arteries in the experiments in panel C were benextramine pretreated. Contraction is expressed as a percentage of the contractile response to 68 mM K+ (% of SD). n=9-11.

The observation that the prazosin - log $K_B$ value was slightly but significantly reduced in benextramine-pretreated, Ang II-exposed tissues, could imply that these treatments introduced the participation of $\alpha_2$ adrenergic receptors in mediating the response to NE. To test this, $-\log K_B$ values for the selective $\alpha_2$-antagonist rauwolscine were determined in femoral arteries pretreated with benextramine and exposed to Ang II. Two concentrations of rauwolscine were used: 1x10^-5 and 3x10^-5 M. Since the $-\log K_B$ values determined at these concentrations were not significantly different, they were pooled, yielding a mean value of 5.52 ± 0.1 (n=16).

It will be argued in the discussion that $\alpha$-adrenoceptor reserve in the rabbit femoral artery attenuated the enhancing affect of Ang II on contractile response to NE. Thus, evidence must be sought that demonstrates the existence of such a reserve. In tissues possessing receptor reserve, irreversible receptor blockade not only reduces the maximal response but shifts the dose-response curve to the right. Comparison of the control NE dose-response curve in Figure 1 to those in Figures 3, 4, and 5 demonstrates that this occurred in the rabbit femoral artery. The threshold concentration of NE in control femoral artery was approximately 3x10^-5 M and that after $\alpha$-adrenoceptor inactivation with benextramine was approximately 1x10^-6 M. To provide more direct evidence for an $\alpha$-adrenoceptor reserve in femoral artery, the method of Furchgott was used to determine the dissociation constant ($K_d$) for NE. That value was 7.76(±3.7SEM) x 10^-4 M determined from three experiments with four replications per experiment. The NE $K_a$ was then used to determine the relation between contractile response and receptor occupancy by NE (see “Materials and Methods”). Figure 7 represents this relation using data from control (Figure 1) and benextramine-pretreated (Figure 5B) femoral arteries. In the control arteries, a very large receptor reserve existed in both the presence and absence of Ang II. For example, 50% of maximal responses were obtained at 0.2% and 0.5% receptor occupancy, respectively. In contrast, there was no receptor reserve after benextramine pretreatment and...
NE behaved as a weak partial agonist. However, there was a much greater difference in receptor occupancy in the presence compared with the absence of Ang II. For example, 10% of maximal contraction was obtained with 8% receptor occupancy in the presence of Ang II and 80% in its absence.

Discussion

In the present study, Ang II enhanced the contractile response of the rabbit femoral artery to NE. In untreated vessels, the enhancement manifested as a small but significant leftward shift of the dose-response curve with no change in the maximal response. After pretreatment with benextramine to substantially reduce the response to NE, Ang II caused only a modest leftward shift of the dose-response curve, but markedly increased the contractile response at all NE concentrations with the greatest increase occurring at the highest concentration of NE used (1 × 10⁻⁴ M).

In a previous study from our laboratory,³³ contractile synergism between two vasoconstrictor agents was assessed. When threshold concentrations of each were combined, a far greater than additive contractile response was obtained. However, if full dose-response curves were obtained to one of the agonists in the presence and absence of a threshold concentration of the other, different results were obtained depending on the two agonists used. In the cases of two α-agonists acting on the same receptor, the two dose-response curves converged, demonstrating that the contractile synergism was based on a phenomenon affecting primarily the threshold response. However, when an α-agonist and serotonin were used, acting on different receptors, the dose-response curve to one agonist in the presence of a threshold concentration of the other was shifted to the left in a parallel fashion. The mechanism of enhancement in this case operated throughout the dose-response curve. In the present study, Ang II caused a parallel leftward shift of the NE dose-response curve in untreated femoral artery rings with convergence occurring only at or near maximal response. Furthermore, when benextramine-pretreated femoral artery rings were preexposed to NE to cause a contraction equal to that caused by Ang II, there was no enhancement of the subsequent NE dose-response curve. Thus, the Ang II enhancement was independent of the small contraction induced by Ang II itself and appears to involve a mechanism which operates throughout the dose-response curve.

The calcium channel antagonist, nifedipine, had different effects depending on the experimental condition. This agent modestly reduced the contractile response to NE in both untreated and benextramine-pretreated femoral artery rings (Figures 2 and 5), suggesting that in these instances, only part of the intracellular calcium mediating contraction was derived from calcium entry via calcium channels. Presumably, the remainder was derived from the release of calcium from intracellular storage pools.²⁶ In contrast, the enhancement of the contractile response to NE caused by Ang II was nearly abolished by nifedipine. This implies that the primary mechanism underlying the Ang II–induced enhancement involved an alteration of calcium channel function. The nature of this alteration is unknown. However, there are several possibilities. As was found in the rabbit saphenous vein,³⁴ Ang II could have induced the expression of α₂-receptors which, in turn, would participate in mediating the response to NE. Furthermore, it is also possible that benextramine pretreatment selectively inactivated α₁-receptors leaving a homogeneous population of α₂-receptors. Several investigators have shown that α₂-receptor mediated vasoconstriction is exquisitely sensitive to calcium channel blockade.³⁵–³⁷ Thus, if Ang II and benextramine exhibited these respective actions, this could explain the marked sensitivity of the Ang II amplified response to calcium channel blockade. However, Ang II–induced expression of α₂-receptors is unlikely in the present study. The −log Kᵦ value for prazosin was identical in the presence and absence of Ang II and was within the range for prazosin acting at α₁-receptors.³⁸ Furthermore, we have obtained a nearly identical pA₂ value for prazosin against the α₁-agonist, clonidine, in the presence of Ang II (9.2 ± 0.2, slope = 1.08, n = 8; M.A. Weber and R.E. Purdy, unpublished observations).

It is also unlikely that benextramine selectively inactivated α₁-receptors leaving α₂-receptors intact. First, benextramine is nonselective between α₁- and α₂-receptors. Second, the present results do not reveal the presence of α₂-receptors in benextramine-pretreated, Ang II–exposed artery rings. The prazosin Kᵦ value in such tissues was 8.71. While this value was slightly below the prazosin Kᵦ in untreated tissues, it was above the Kᵦ of prazosin acting at the α₁-receptors of rabbit aorta (8.5³⁸). As a further test, the −log Kᵦ value for the selective α₂-antagonist, rauwolscine, was also determined in benextramine-pretreated, Ang II–exposed femoral arteries. That value, 5.52, agrees with published values for rauwolscine acting at the α₁-receptor.³⁹ Thus, it is likely that neither Ang II nor benextramine pretreatment altered the receptor subtype mediating the response of the femoral artery to NE. Furthermore, the present results suggest that this subtype was α₁.

It is possible that benextramine could have inhibited intracellular calcium release by an α-receptor independent mechanism. Timmermans and coworkers provided indirect evidence that another irreversible α-antagonist, phenoxybenzamine, may possess such an action. If so, this could explain the sensitivity of the Ang II–amplified response to blockade by nifedipine in benextramine-pretreated tissues. However, Timmermans and coworkers failed to demonstrate such an action for benextramine under experimental conditions identical to those in
which phenoxylbenzamine was used. Furthermore, the Ang II–induced enhancement of response to NE was abolished by nifedipine in both untreated and benextramine-pretreated vessels. Thus, sensitivity to calcium channel blockade was independent of benextramine pretreatment.

That nifedipine nearly abolished the Ang II–amplified response to norepinephrine, points to a possible mechanism for the amplifying action of Ang II. This agent could have increased the efficiency of coupling of α-receptor activation and calcium channel opening. In a seminal study, Lues and Schumann40 have proposed such a mechanism in the rabbit thoracic aorta. These authors found that BHT-920, an α-selective agonist in vivo, not only failed to cause a contraction, but behaved as an α1-antagonist in the aorta. On the other hand, BHT-920 became a partial α1-agonist in the presence of subthreshold and threshold concentrations of Ang II. These authors suggested that Ang II amplified the response to BHT-920 by “... influencing calcium channels which link the [alpha] receptors to the chain of processes through which the contraction of the cell is triggered.” 39

The amplification phenomenon described by Lues and Schumann39 in the present study may not involve a specific interaction between angiotensin receptors and alpha receptors. Lues and Schumann39 observed that reserpine pretreatment, ouabain, serotonin, and prostaglandin F2α also caused BHT-920 to behave as a partial alpha agonist. In addition, Day and Moore36 found that Ang II enhanced the contractile response of rabbit aortic strips to several agonists including NE, potassium chloride, histamine, acetylcholine, isoprenalin (acting as an α-agonist) and serotonin.

The facilitatory effect of Ang II on the neuronal release of NE and on effector cell responsiveness in cardiovascular tissues is antagonized by the synthesis of arachidonic acid metabolites.40,41 This raises the possibility that prostaglandins could have played a role in the observations of the present study. No experiments were carried out to test this. However, Forstermann and coworkers42 studied several properties of the prostaglandins in the rabbit femoral artery. They found that exogenous prostaglandins E2 and F2α enhanced contractility to NE. However, prostaglandin F2α is not synthesized by the femoral artery, and prostaglandin E2 synthesis can be induced by 3 × 10−8 M arachidonic acid, but not by either 3 × 10−8 Ang II or 3 × 10−7 NE. The observations of Forstermann and coworkers suggest that prostaglandins are unlikely to account for the amplifying effects of the more than 30-fold lower concentration of Ang II used in the present study.

It is argued below that the presence of a high α-adrenoceptor reserve in the rabbit femoral artery attenuated the enhancing effect of Ang II on the contractile response to NE. To support this argument, it was necessary to establish that such receptor reserve existed. Femoral artery rings were studied using the method of Furchgott37, and receptor reserve was considered to exist if less than 50% of available receptors were occupied by NE at 50% of maximal contractile response.32 According to this criterion, the femoral artery possessed a substantial reserve of α-adrenoceptors. For example, NE elicited a 50% of maximal contractile response occupying only 0.5% of available receptors in untreated femoral arteries (see Figure 7). In contrast, NE could only elicit between 20% and 50% of the maximal control response after receptor inactivation with benextramine and this required 100% receptor occupancy. In addition, the 50% of maximal response achieved in benextramine-pretreated vessel segments required the occupation of at least 50% of the available α-receptors. Thus, an α-adrenoceptor reserve existed prior to but not after benextramine pretreatment.

Several authors have observed that noncompetitive blockade of vasoconstrictor response by calcium channel antagonists,29 organic nitrates,30 and cooling31 was attenuated under conditions of high receptor reserve. This was explained in terms of the relation between stimulus and receptor occupancy by agonist.31 Noncompetitive blockade increases the stimulus required to reach threshold for contraction. However, in the presence of a large receptor reserve, the fraction of receptors required to deliver this additional stimulus is small, and, therefore, the increase in agonist concentration required to occupy the additional fraction of receptors is also small; that is, receptor reserve buffers noncompetitive blockade. In the absence of receptor reserve, a much larger fraction of receptors must be occupied to deliver the same additional stimulus and, thus, a substantially greater increase in agonist concentration is required. In the present study, enhancement, not inhibition, was observed, and the magnitude of enhancement was increased by the elimination of receptor reserve. Ang II decreased the stimulus required for a given level of contractile response to NE. However, the presence of the high receptor reserve in the femoral artery meant that the change in receptor occupancy corresponding to the change in stimulus was small. In contrast, after benextramine pretreatment, the Ang II–induced decrease in the required stimulus corresponded to a greater reduction in fractional receptor occupancy and, therefore, in the agonist concentration required to elicit a given response.

Several questions must be answered in order to establish that receptor reserve did attenuate the magnitude of enhancement of contractility by Ang II: 1) Was the reduction of stimulus required for a given level of contractile response constant in both control and benextramine-pretreated tissues? 2) Did this constant stimulus correspond to different levels of receptor occupancy before and after receptor inactivation? 3) How does receptor reserve “buffer” noncompetitive blockade or enhancement? 4) How might such
buffering alter hemodynamic function in vivo? These questions are addressed in the following discussion.

It is likely that Ang II decreased the stimulus required for a given level of contractile response by a constant amount in both control and benextramine-pretreated tissues. This is based, in part, on the likelihood (see above) that Ang II acted by sensitizing calcium channels rather than by exerting some direct effect on α-adrenergic receptors. Thus, benextramine pretreatment, inactivating only alpha receptors, would not alter Ang II receptor function (the magnitude of Ang II–induced threshold contraction was the same in both control and benextramine-pretreated tissues). It is also based on a central assumption of pharmacological receptor theory, namely, that occupation of equal numbers of receptors always yields the same magnitude of response; that is, delivers the same stimulus. This assumption is justified since a single concentration of Ang II was used in all experiments of the present study, 3 x 10^-10 M, and since it is unlikely that any of the experimental procedures altered either Ang II receptor affinity, density, or efficiency of coupling to contraction.

While the effect of Ang II on stimulus required for a given level of contractile response was likely constant, the corresponding fractions of receptors occupied by NE before and after receptor inactivation differed markedly. For example, comparing at 10% of maximal contraction (Figure 7), 0.6% and 0.2% of receptors were occupied by NE in the absence and presence of Ang II in control tissues. This corresponded to a very small difference in NE concentrations, 4.6 x 10^{-9} and 1.2 x 10^{-9} M, respectively. In contrast, after benextramine pretreatment, 80% and 8% of receptors were occupied by NE in the absence and presence of Ang II. This corresponded to a much larger difference in NE concentrations, 3 x 10^{-5} and 6 x 10^{-7} M, respectively.

The nature of the buffering effect of receptor reserve is best understood in terms of the family of curves generated by changing efficacy. In the case of high efficacy, the dose-response curve is obtained at very low agonist concentrations. Reducing efficacy shifts the curve to the right as long as receptor reserve exists and ultimately causes a reduction of maximal response when receptor reserve is exhausted. In order to visualize this, we have generated a series of dose-response curves for epinephrine and clonidine in the rabbit ear artery (Figure 8).

The first curve for each agonist is the actual curve obtained experimentally in an earlier study.22 The subsequent curves represent 25%, 50%, 75%, and 90% reductions in efficacy, calculated using experimentally determined K⁎ values, fractions of receptors occupied at given levels of contraction and Mass Law assumptions. It can be seen in Figure 8 that changing efficacy in the case of epinephrine, a highly efficacious full agonist, caused small parallel shifts of the dose-response curve. In contrast, in the case of clonidine which was a partial agonist with low efficacy, changing efficacy resulted primarily in marked changes in maximal contractile response. These theoretical curves very closely resemble the changes produced by Ang II in the NE dose-response curves of the present study. In control tissues (Figure 1), Ang II produced a small, parallel leftward shift, consistent with the present finding that these tissues possess a large receptor reserve. In benextramine-pretreated tissues, lacking receptor reserve, Ang II caused a marked upward shift of the NE dose-response curve. While these comparisons do not constitute proof, they are consistent with our conclusion that receptor reserve attenuated the magnitude of the enhancement by Ang II in the control tissues.

The impact of the enhancing effect of Ang II on hemodynamics in vivo is difficult to predict from the present results. However, some insight may be gained by subtracting the dose-response curves in the absence of Ang II from those in its presence. This yields the magnitude of the enhanced response by itself. As seen in Figure 9, in control tissues, the greatest Ang II–induced increase in contractile response occurred at 1 x 10^{-8} M NE and represented approximately 23% of the maximal response to 68 mM K⁹. The degree of enhancement by Ang II in benextramine-pretreated tissues was not markedly different. The greatest amplification occurred at 3 x 10^{-8}M, representing approximately 33% of the maximal response to 68 mM K⁹. In both cases, much of the Ang II–induced enhancement occurred within a 100-fold NE concentration range. At first glance, this comparison suggests that enhancement of contractility by Ang II will not be very different in a vascular bed with high receptor reserve compared to one with little or no receptor reserve. However, we suggest that the enhancement by Ang II is best understood in terms of the inherent capacity of each blood vessel or vascular bed to

![Figure 8. Families of epinephrine (EPI) and clonidine (CLO) dose-response curves in the rabbit ear artery. The first curve for each agonist is the actual curve obtained experimentally in an earlier study22 and the remaining curves are theoretical, reflecting the effects of reducing (RE) receptor reserve by 25%, 50%, 75%, and 90%. (See text for further discussion.)](image-url)
contract. In control femoral artery rings, the maximum Ang II-induced enhancement represented 20% of that capacity (Figure 1). In contrast, it represented between 200% and 800% of the inherent capacity of benextramine-pretreated vessels to contract (Figure 5B). Extrapolating these findings to the in vivo setting, vascular beds with high receptor reserve will exhibit a modest enhancement of the response to NE in the presence of Ang II, and the inherent capacity to contract will not be changed. This may be reflected as a slightly increased resistance to flow. However, in vascular beds with no receptor reserve, the inherent capacity to contract will be increased up to eightfold; that is, in the presence of Ang II, these vascular beds become much more reactive to any concentration of NE. Thus, in the presence of Ang II, NE is likely to produce a marked increase in resistance to flow in such vascular beds.

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R E Purdy and M A Weber

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