Inhibition of Angiotensin II Potentiation of Sympathetic Nerve Activity by Beta-Adrenergic Antagonists

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SUMMARY Since β-adrenergic blockers are effective in the therapy of hypertension by a mechanism related to the degree of activation of the renin-angiotensin system, the effect of eight β blockers was examined on angiotensin II potentiation of nerve stimulation (NS) in isolated perfused rat mesenteric vessels. The vasoconstrictor response to periartrial NS was obtained by monitoring changes in perfusion pressure while a β blocker or a β blocker and angiotensin II (3 ng/ml) were added to the perfusate. Although each β blocker tended to decrease responses to NS, in the concentrations used, only metoprolol significantly inhibited responses to NS. Angiotensin II, when infused alone, potentiated the responses to NS by 63% (p < 0.01). These enhanced responses following angiotensin II were inhibited in a dose-related manner (10–300 ng/ml) by β, β, and mixed β blockers. At the 100 ng/ml concentration, DL-propranolol, timolol, metoprolol, practolol, butoxamine, and H35/25 inhibited the angiotensin II potentiation of NS by 83%, 76%, 77%, 59%, 72%, and 41% respectively. The order of potency for this action was as follows: timolol = metoprolol = butoxamine > propranolol > practolol > H35/25. Administration of D- and L-propranolol also reduced the responses by 75%. The vasoconstrictor responses to injected norepinephrine (NE), in the presence and absence of angiotensin II, were not altered by DL-propranolol or timolol. In conclusion, β-adrenergic blockers were found to interfere with the effect of angiotensin II on the sympathetic neuron, a property that could contribute to the antihypertensive action of these drugs. (Hypertension 2: 90–96, 1980)

KEY WORDS • beta-adrenergic blockers • angiotensin II • sympathetic nervous system • nerve stimulation • renin-angiotensin system

BETA-ADRENERGIC antagonists are effective in the treatment of human essential hypertension. This antihypertensive effect appears to be related to the level of activity of the renin-angiotensin system since lowering of blood pressure is most pronounced in those patients with high plasma renin activities1-4 and hence higher-than-normal plasma angiotensin II levels. The effectiveness of β blockers in lowering blood pressure in these hypertensive patients has been attributed primarily to the ability of the drugs to decrease the release of renin and decrease cardiac output.1,4

Additionally, recent studies indicate that the antihypertensive effect of β blockers is also related to the level of activity of the sympathetic nervous system in patients with high-renin essential hypertension.4 An analogous situation has been observed in experimental animals. Chronic intravenous infusions of angiotensin II in subpressor doses will produce a gradual rise in blood pressure.6,7 Since adrenergic neuron-blocking drugs can lower the blood pressure to within normal range in these hypertensive animals,5,6 this form of experimental hypertension is thought to be due to an increase in sympathetic tone. Thus, by similar mechanisms, the plasma angiotensin II levels associated with normal or high plasma renin activities may produce hypertension by enhancing sympathetic tone, either by a central effect on the vasomotor center11,12 or by a peripheral effect on the sympathetic neuron,13-15 or both.

Since we have previously found that angiotensin II in subpressor concentrations will potentiate the vasoconstrictor responses to sympathetic nerve stimulation (NS),16 we initiated this investigation to test the hypothesis that β-adrenergic blockers reduce vascular tone by interfering with the actions of angiotensin II on the peripheral sympathetic neuron. Utilizing the isolated perfused rat mesentery, we ex-
amined the effect of eight β-adrenergic blockers on the vasconstrictor responses to sympathetic NS in the presence and absence of angiotensin II. Additionally, the effects of β blockade were also examined on the responses to exogenous norepinephrine to determine if the site of action was pre- or postsynaptic.

Methods

Male Sprague-Dawley rats (Simonsen Labs) weighing 200–300 g were anesthetized with pentobarbital (50 mg/kg, i.p.). The superior mesenteric artery was isolated, cannulated, and removed with its small resistance vessels, as described by McGregor. The mesenteric arterial vessels were then perfused with a modified Krebs' solution of the following composition (in mM): NaCl 112, KCl 5.0, NaH2PO4 1.0, MgCl2 0.5, CaCl2 2.5, NaHCO3 25.0, D(+)-glucose 11.2. The perfusate was aerated with a 95% O2 and 5% CO2 mixture and maintained at 30°C. The pH and osmolarity of the perfusate were 7.4 and 284 mOsm respectively. A Harvard peristaltic pump (model 1210) was used to perfuse the mesenteric vessels at a constant rate of 10 ml/min, while changes in perfusion pressure were monitored with a Narco RP-1500 pressure transducer and recorded on a Narco Physiograph (model DMP-4B). The average mean pressure in the perfusion cannula before cannulation of the superior mesenteric artery was 5.0 ± 0.3 mm Hg (n = 10), and the average mean pressure after cannulation was 34.1 ± 1.8 mm Hg (n = 10).

Once perfusion of the mesenteric vessels was begun, 20–60 minutes were allowed for stabilization of basal perfusion pressure. Sympathetic NS was accomplished by placing a bipolar platinum electrode around the superior mesenteric artery and stimulating with a Narco SI-10 stimulator at 4-minute intervals at 11 Hz for 20 seconds using 12V biphasic rectangular pulses 2 msec in duration. Abolition of response to NS by phenoxybenzamine administration, 6-hydroxydopamine pretreatment, and reserpine pretreatment verified that periarterial stimulation was mediated by stimulation of sympathetic nerve fibers and not by direct vascular smooth-muscle stimulation. Responses to norepinephrine (NE) were produced by injecting 20 μl of normal saline containing 200 ng of NE into an injection port proximal to the perfusion pump. Changes in mesenteric vascular resistance due to NS and NE were measured as an increase in perfusion pressure in this constant flow system. As shown in table 1, the vasoconstrictor responses produced by 200 ng NE or by 11 Hz NS were of similar magnitude. Additionally, these stimulation parameters provided responses to NE and NS that were approximately within the same region of their respective dose-response and frequency-response curves, i.e., the lower extremity of the linear region.

Only one experiment was performed per mesenteric preparation, according to the following protocol. After four responses to either NS or NE (but not both) were obtained, a β blocker or its vehicle (Krebs' solution) was infused (0.1 ml/min) into the perfusate at a concentration sufficient to provide a final perfusate concentration of 0, 10, 30, 100, or 300 ng/ml. Four more responses to the same stimulus were obtained 15 minutes later. While continuing the β blocker or vehicle infusion, we began an infusion of angiotensin II (dissolved in Krebs' solution) to provide a final perfusate concentration of 3 ng/ml, a concentration that was subpressor in our system. After 15 minutes of infusing angiotensin II plus a β blocker or angiotensin II plus vehicle, we obtained four more responses to either NS or NE. The results were evaluated by comparing the mean response to NS or NE before infusion of any drugs to those obtained after the infusion of a β blocker alone or a β blocker in combination with angiotensin II. These results were then expressed as the percent of the control response for each experiment (experimental/control X 100). For each β blocker at each concentration, the values from six experiments were combined to give a mean and standard error. Since the results are presented as percent of control, a value greater than 100% would indicate an enhanced response while a value less than 100% would represent an inhibition. The drugs and their sources of supply are as follows: angiotensin II (Beckman), norepinephrine HCl (Sigma Chemical), DL-propranolol HCl (Ayerst), D-propranolol (Ayerst), L-propranolol (Ayerst), timolol maleate (Merck Sharp and Dohme), practolol (Ayerst), bimetoprolol tartrate (CIBA), H35/25 (Hassle), and butoxamine HCl (Burroughs Wellcome).

Statistical analysis was performed by utilizing a Mann-Whitney U test since percentages follow a binomial rather than a normal distribution. The percentage change in response to NS or NE during vehicle infusion (i.e., 0 dose of β blocker) was compared to the percentage change in response during a β blocker infusion.

Results

Two non-selective β-adrenergic blockers, DL-propranolol and timolol, caused a slight, non-significant decrease in response to NS (fig. 1). Krebs' solution, when infused alone (i.e., 0 ng/ml concentration of β blocker), resulted in a slight decrease in the response to NS which was due to a gradual change in responses with time. This influence of duration of perfusion necessitated comparing the results obtained
after β-blocker treatment with those obtained after vehicle infusion. After treatment with angiotensin II in the absence of a β blocker, the responses to NS were 63% above control \( (p < 0.01) \), indicating a significant enhancement of the vasoconstrictor responses. At 30 ng/ml concentration, DL-propranolol had no effect on this potentiated response to NS, but at 100 ng/ml, it significantly reduced this enhanced response by 83% \( (p < 0.05) \). Similarly, timolol inhibited angiotensin II potentiation of NS by 34%, 70% \( (p < 0.05) \), and 76% \( (p < 0.05) \) at 10, 30, and 100 ng/ml concentrations respectively.

Further studies were conducted using relatively selective β₁-adrenergic blockers. As depicted in figure 2, practolol had little effect on responses to NS, yet decreased the enhancement of NS by angiotensin II by 44% and 59% \( (p < 0.05) \) at the 30 and 100 ng/ml concentrations respectively. Metoprolol, another β₁-selective blocker, at 100 and 300 ng/ml significantly diminished the response to NS and blocked the enhancement of NS by angiotensin II by 77% \( (p < 0.05) \) and 75% \( (p < 0.05) \) respectively.

Figure 3 summarizes the results of experiments performed with relatively selective β₂-adrenergic blockers. At both 100 and 300 ng/ml concentrations, H35/25 had little effect on NS and only the 300 ng/ml concentration caused a small, nonsignificant decrease in the enhancement of NS by angiotensin II. On the
other hand, butoxamine decreased the response to NS and inhibited the enhancement of NS by angiotensin II by 72% (p < 0.05) at both the 100 and 300 ng/ml concentrations.

Additional experiments were conducted with the D- and L-stereoisomers of propranolol to determine if the inhibition of angiotensin II enhancement of NS by β blockers was stereospecific. As shown in figure 4, both D- and L-propranolol at 50 ng/ml inhibited the enhancement of NS by angiotensin II by 75% (p < 0.05) without significantly altering the response to NS in the absence of angiotensin II.

Finally, we examined the effects of DL-propranolol and timolol on response to exogenous NE in the presence and absence of angiotensin II (fig. 5). The response to NE increased slightly with time, as indicated by the positive slope of the line. Angiotensin II, when infused alone, potentiated the response to NE by 62% (p < 0.01). Neither DL-propranolol nor timolol altered the responses to NE or the potentiation of NE responses produced by angiotensin II.

**Discussion**

In these studies, β-adrenergic blockers have little effect on vasoconstrictor responses to NS in the concentrations employed. Angiotensin II enhances the vasoconstrictor responses to both NS and NE by 63% and 62% respectively. Neither NE-induced vasoconstriction nor angiotensin II potentiation of NE responses are affected by β-blocking agents. On the other hand, β-nonselective, β-selective, and β₁-selective adrenergic blockers inhibit the potentiation
by angiotensin II of the vasoconstrictor responses to sympathetic NS. The order of potency for this action of β-blockers is: timolol = metoprolol = butoxamine > propranolol > practolol > H35/25.

As shown in figure 5, β-adrenergic blockers fail to alter the responses to exogenous NE or the potentiation of NE responses by angiotensin II. These observations indicate that the β blockers do not exert their action by interfering with the postsynaptic vasoconstriction produced by NE. Additionally, since β blockers selectively inhibit angiotensin II enhancement of NS without affecting angiotensin II enhancement of NE responses, angiotensin II must be enhancing the responses to NS and NE by different presynaptic mechanisms. Angiotensin II probably enhances NE responses by inhibiting neuron uptake of NE,16-19 whereas angiotensin II potentiates response to NS by enhancing the release of NE.18-19 Thus, it appears that β blockers inhibit the ability of angiotensin II to enhance the release of NE following sympathetic NS, without affecting the ability of angiotensin II to block neuron reuptake of NE.

Several investigators20,21 have proposed that presynaptic β-adrenergic receptors are located on the sympathetic neuron and that they enhance the neuron's release of NE. According to these authors, the NE released following NS will activate these presynaptic β receptors, which in turn will stimulate further NE release. Beta-adrenergic blockers inhibit this effect. Similarly, other investigators have postulated that presynaptic angiotensin II receptors also enhance the release of NE following NS, an effect that angiotensin antagonists block.13-16,22 Interestingly, in our present study, β-adrenergic blockers inhibit the enhanced responses and apparently the enhanced NE release that angiotensin II produces following NS. Although the β blockers may exert this inhibitory effect by a mechanism unrelated to β-adrenergic receptor blockade (see below), these data may be interpreted to suggest the existence of a functional relationship between the presynaptic β-adrenergic and angiotensin II receptors. Along these lines, our previous finding that propranolol inhibits saralasin-induced renin release suggests that a relationship also exists between the juxtaglomerular cell β-adrenergic receptor and blockade of the inhibitory "short loop" by which angiotensin II inhibits renin release.23

At present, the mechanism by which β-adrenergic blockers inhibit the potentiation of NS by angiotensin II has not been identified. Both D- and L-stereoisomers of propranolol demonstrate the capacity to inhibit angiotensin II enhancement of sympathetic nerve transmission (figure 4), even though only the L-stereoisomer has been reported to have significant β-blocking activity.24 Hence, β blockade is apparently not a requirement for inhibition of the action of angiotensin II on the sympathetic neuron. Alternatively, it may be that β blockade is required but the presynaptic β receptor that mediates this effect lacks stereospecificity. However, in view of the steep dose-response relationship for the abolishment of angiotensin II potentiation by various β blockers, a competitive antagonism of presynaptic β receptors seems unlikely. It should be mentioned that the antihypertensive effect of D-propranolol has not been examined in doses sufficient to provide plasma levels of 50 ng/ml or above.25 In the absence of this information, we must question the belief currently held that D-propranolol is devoid of antihypertensive activity.

Beta blockers devoid of membrane-stabilizing activity (practolol, timolol, and metoprolol) are as effec-
tive in inhibiting angiotensin II potentiation of NS as are β blockers with membrane-stabilizing activity (propranolol, butoxamine). Also, the concentrations used in these studies are much less than those required for membrane stabilization. Thus, the ability to stabilize membranes cannot account for the effects of β blockers on angiotensin II-induced enhancement of NS. Finally, the degree to which the β blockers possess intrinsic sympathomimetic activity does not correlate with their ability to inhibit the potentiation of NS by angiotensin II. Moreover, sympathomimetics would be expected to enhance rather than inhibit the release of NE.

Several investigators have reported that propranolol is an adrenergic neuron blocking agent. The possibility, that the inhibitory effect of the β blockers on the potentiation of NS by angiotensin II is related to neuron blockade, should be considered. However, two lines of evidence tend to refute such a hypothesis. First, most studies demonstrate adrenergic neuron blockade by propranolol at concentrations of 500–14,000 ng/ml, whereas we find that propranolol inhibits the angiotensin II enhancement of NS at a much lower concentration, 100 ng/ml. Second, we fail to observe a relationship between inhibition of responses to NS and inhibition of angiotensin II enhancement of NS. Thus, it appears unlikely that the adrenergic neuron blocking action of these drugs is responsible for their effects on angiotensin II enhancement of NS.

As previously mentioned, the enhancement of sympathetic nerve activity by angiotensin II may contribute to the development and maintenance of some forms of hypertension. Several studies by Fernandes et al. support this view. In rats made hypertensive by coarctation of the aorta between the renal arteries, a 25-minute infusion of saralasin (an angiotensin II antagonist) lowers blood pressure markedly during the early phases of hypertension, yet has little effect on blood pressure during the chronic phase. In our laboratory, angiotensin antagonists rapidly reverse the direct vascular effects of angiotensin II, whereas the reversal of angiotensin II potentiation of sympathetic nerve activity requires 1 hour of antagonist infusion (unpublished observation). Zimmerman reports a similar delay in the onset of antagonism by saralasin potentiation of NS in the dog hindpaw.

Thus, in the study above, the acute elevation of blood pressure appears to be due to the direct vascular effects of angiotensin II, while the chronic elevation is not. However, chronic elevation of blood pressure may be the result of angiotensin II enhancement of sympathetic nerve activity. This contention is suggested by the finding that if rats are chemically sympathectomized with 6-hydroxydopamine before aortic coarctation, the acute rise in blood pressure is not affected, but the chronic rise in blood pressure is significantly reduced. Interestingly, propranolol reduces somewhat both the acute and chronic rise in blood pressure that follows aortic coarctation. Thus, it is tempting to speculate that the antihypertensive action of propranolol may be explained in the early phases of aortic coarctation hypertension by suppression of renin release, but in the chronic phase by an inhibition of the enhanced sympathetic nerve activity produced by angiotensin II.

In another study, Ljung et al. report that blood pressure and the portal vein response to transmural stimulation are decreased in spontaneously hypertensive rats treated chronically with propranolol or metoprolol, compared with untreated rats. The portal vein responses to NE are similar in the three groups. Thus, one may suggest that the β blockers lower blood pressure in this form of normal-renin hypertension by reducing the activity of the sympathetic nervous system peripherally.

As shown in table 2, the concentration of each β blocker required to inhibit angiotensin II enhancement of sympathetic NS correlates well with the plasma levels achieved by therapeutic doses of these drugs. The fact that antagonism of angiotensin II enhancement of NS occurs at therapeutic concentrations suggests that this mechanism may contribute to the antihypertensive action of β blockers. It should be emphasized, however, that these studies suggest, but do not prove, such a hypothesis.

In conclusion, β-adrenergic blockers can inhibit the potentiation of sympathetic NS by angiotensin II. This effect appears to be due to a presynaptic action of the β blocker; but neither β-adrenergic blockade, intrinsic sympathomimetic activity, nor membrane stabilization can satisfactorily explain this effect. Nonetheless, a portion of the antihypertensive effect of β-adrenergic blockers may be attributed to an interference with the effects of angiotensin II on the peripheral sympathetic neuron. Furthermore, this effect of the β blockers on the sympathetic neuron, combined with their ability to suppress renin release, may explain the relationship between the level of activity of the renin-angiotensin system and the antihypertensive activity of β-adrenergic blockers.

Table 2. Comparison Between Plasma Concentration of Several Beta Blockers Required for Antihypertensive Activity and Concentration Required to Inhibit Angiotensin Enhancement of Nerve Stimulation

<table>
<thead>
<tr>
<th>β blocker</th>
<th>Therapeutic dose range (mg/day)</th>
<th>Plasma concentration at minimal therapeutic dose (ng/ml)</th>
<th>Concentration that inhibits AII enhancement of NS (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol</td>
<td>160–320</td>
<td>40^4</td>
<td>50</td>
</tr>
<tr>
<td>Timolol</td>
<td>20–40</td>
<td>55^7</td>
<td>30</td>
</tr>
<tr>
<td>Prazolol</td>
<td>200–1200</td>
<td>110^6</td>
<td>100</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>160–400</td>
<td>113^6</td>
<td>100</td>
</tr>
<tr>
<td>Butoxamine</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>H35/25</td>
<td>—</td>
<td>—</td>
<td>300</td>
</tr>
</tbody>
</table>

Superscripts refer to references; AII = angiotensin II; NS = nerve stimulation.
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