Clonidine Reverses the Slowly Developing Hypertension Produced by Low Doses of Angiotensin II

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Abstract We used the centrally acting sympatholytic drug clonidine to evaluate neurogenic pressor activity in rats made hypertensive by chronic intravenous infusion of a low dose (4.0 ng · min⁻¹) of angiotensin II (Ang II). Male Sprague-Dawley rats were instrumented with arterial and venous catheters and then housed in metabolic cages for the duration of the experiment. After 3 days of recovery from surgery, daily measurements were begun of mean arterial pressure, heart rate, water balance, and urinary electrolyte excretion. After 3 days of control measurements rats received either Ang II (4.0 ng · min⁻¹ IV, n=5) or saline vehicle (n=4) continuously over a 15-day period. After the Ang II and vehicle infusions were ended, measurements were made during 3 days of recovery. On days 2, 7, and 12 of the experimental infusion period, clonidine hydrochloride was administered as a bolus (10.0 µg · kg⁻¹ IA). The resulting changes in mean arterial pressure and heart rate were assessed over a 6-hour period. Fluid measurements were evaluated on a daily basis. In rats receiving only vehicle, clonidine produced no significant changes in any variable at any time. In rats given Ang II, mean arterial pressure increased from a control value (mean±SEM) of 106±1 mm Hg to 135±6, 139±6, and 148±4 mm Hg on days 2, 7, and 12, respectively. The antihypertensive response to clonidine after 6 hours on days 2, 7, and 12 of the Ang II infusion in these rats was -18±8, -16±5, and -23±9 mm Hg, respectively. This effect was statistically significant on all days of the Ang II infusion. No other variable was affected by clonidine. These results support the theory that circulating Ang II increases arterial pressure in part by an action on the sympathetic nervous system. (Hypertension. 1994;23[part 2]:844-847.)

Key Words: angiotensin II · hypertension, experimental · clonidine · renin-angiotensin system

An important role for the renin-angiotensin system (RAS) in the pathogenesis of renovascular hypertension in humans and experimental animals is well established. However, Robertson and colleagues have proposed that three reasonably distinct temporal phases of RAS involvement in renovascular hypertension can be identified. In phase I, plasma angiotensin II (Ang II) concentrations are markedly elevated, sufficient to cause direct vasoconstriction. In phase II, plasma Ang II is normal or only slightly elevated, but blood pressure can still be reduced by inhibitors of Ang II formation or by relief of the renal artery stenosis. In phase III, hypertension is no longer related to RAS activity. Most human patients diagnosed with renovascular hypertension are in phase I or II.

The ability of normal plasma concentrations of Ang II to support elevated blood pressure in phase II has been puzzling. Some investigators have proposed a role for specific nonrenal tissue RASs (eg, vascular, brain, adrenal) in this situation, whereas others have called attention to the "slow pressor" effect of circulating Ang II. It has long been known that Ang II, when infused intravascularly at quite a low dose (initially subpressor), can gradually produce marked hypertension. This slow pressor effect has been postulated to be responsible for the clear dependence of phase II renovascular hypertension on the RAS in the absence of substantially increased plasma Ang II concentrations.

The mechanism of the slow pressor effect of Ang II has been ascribed to renal sodium retention, sympathoexcitation, and most recently, structural cardiovascular changes produced by Ang II. Past studies from this laboratory using long-term intravenous infusions of low doses of Ang II in chronically instrumented rats indicated that the neural actions of Ang II are a key part of the slow pressor effect. Evidence included a gradually developing augmentation of the depressor response to ganglion blockade in rats receiving chronic Ang II infusions and elimination of the slow pressor effect by ablation of the area postrema, a brain region known to mediate some of the central actions of circulating Ang II.

The centrally acting sympatholytic drug clonidine has been shown to cause a dramatic reduction in blood pressure without lowering plasma renin activity in humans with renovascular hypertension. This result was interpreted to indicate a neurogenic basis for phase II renovascular hypertension in humans and may also indicate operation of the slow pressor effect in such patients. The current experiment was performed to determine whether clonidine could in fact reverse the slow pressor effect of Ang II.

Methods

Animals and Protocols

Male Sprague-Dawley rats (Sasco King) weighing 350 to 400 g were used in the studies. Before arterial and venous catheterizations the animals were housed in plastic cages in temperature- and humidity-controlled light-cycled rooms. They received tap water and standard rat chow (Purina Mills). All the animals were surgically prepared and housed chronically.
The experimental protocols were started 3 days after catheterization of the rats. Mean arterial pressure (MAP) and heart rate (HR) were recorded daily throughout the experimental protocol for 10 to 30 minutes between 8 AM and noon by connecting the exteriorized arterial line to a pressure transducer (model P50, Gould Instruments) attached to a blood pressure monitor (model BP2, Steinke Inc). Distilled water was provided ad libitum in calibrated drinking tubes for measurement of daily water intake. Urine output was measured by collection of urine into calibrated cups. Water balance was calculated as the difference between water intake and urinary output. Urine samples were collected daily for determination of urinary sodium excretion using a photometric electrolyte analyzer (model 943, Instrumentation Laboratories Inc).

One group of rats (n=5) received Ang II for 15 days at a dose of 4 ng·min⁻¹, and another group (n=4) received saline. Control period measurements were obtained for 3 days before Ang II or vehicle infusion. On days 2, 7, and 12 of the Ang II or saline infusion, clonidine hydrochloride at a rate of 5.0 mL·d⁻¹ for the entire protocol. It has been previously shown that this moderately high sodium intake for rats accelerates hypertension development during Ang II infusion.²⁻¹¹

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Statistical Analysis
Analyses were performed initially with repeated-measures ANOVA. Post hoc comparisons of means were made with the Student-Newman-Keuls test. The effects of clonidine were analyzed with a two-way mixed-design ANOVA followed by analysis of simple main effects. A probability value of less than .05 was the criterion for statistical significance. All data are expressed as mean ± SEM.

Results
Infusion of Ang II caused a significant increase in MAP throughout the 15-day infusion period (Fig 1). There were no associated changes in HR, water balance, or sodium excretion. Termination of Ang II infusion caused an immediate return (within 24 hours) of MAP to the control period level. Rats not receiving Ang II showed no significant change in any measured variable (Fig 2).

The results of bolus injections of clonidine at a dose of 10 μg·kg⁻¹ into animals receiving chronic intravenous infusion of Ang II at 4 ng·min⁻¹ (n=5) are illustrated in Fig 1. Clonidine lowered MAP on day 2 of the Ang II infusion period, and the decrease at 6 hours (−18±8 mm Hg) was statistically significant (P=0.03). Similar results were obtained on day 7, when by 6 hours clonidine had significantly decreased MAP by 16±5 mm Hg (P=0.05). Clonidine produced a fall in blood pressure on day 12 of the Ang II infusion that was statistically significant at both 2 (−18±3 mm Hg, P=0.03) and 6 (−23±9 mm Hg, P=0.008) hours after clonidine administration. MAP returned to preclonidine values within 24 hours after drug injection. HR was not consistently affected by bolus injection of clonidine on day 2, 7, or 12 of the Ang II infusion. Water balance and urinary sodium excretion also were not significantly affected by clonidine. In rats receiving only saline vehicle (Fig 2), clonidine did not cause a significant change in any variable on any of the 3 days of administration.
Discussion

A variety of pharmacologic tools have been used in the past to estimate the neurogenic component of arterial pressure regulation in hypertension. Foremost among these are ganglionic blockers and α-adrenergic receptor antagonists. In experimental hypertension caused by chronic low-dose infusions of Ang II, both of these types of agents have been shown to elicit an exaggerated fall in arterial pressure relative to that observed in normotensive animals. This has been taken to support the hypothesis that Ang II-induced hypertension has a substantial neurogenic basis. A complication inherent in this approach is that ganglionic blockers and α-adrenergic receptor antagonists lower blood pressure in normotensive animals. Therefore, the enhanced depressor activity of these agents in hypertensive animals might be explained as a nonspecific augmentation of vasodilation expected in any hypertensive individual—the so-called "vascular amplification" property of the hypertensive circulation.

Clonidine is an α₂-adrenergic receptor agonist that readily penetrates the central nervous system and causes a decrease in sympathetic activity. This effect may result from stimulation of α₂-adrenergic receptors or from activation of recently characterized imidazole-preferring receptors. Clonidine may act at several brain regions to cause sympathoinhibition, but the rostral ventrolateral medulla—where cell bodies of many bulbospinal neurons impinging on sympathetic preganglionic neurons are located—seems the most important. The drug lowers arterial pressure in diverse types of clinical and experimental hypertension, but at lower doses it has little or no effect on pressure in normotensive individuals. This property gives clonidine a distinct advantage over other sympatholytic agents for identifying abnormalities of sympathetic function contributing to the pathogenesis of hypertension, as discussed above.

In the present experiment a parenteral dose of clonidine was used that is known to cause sympathoinhibition and a depressor effect in some forms of experimental hypertension in rats. Injection of clonidine into conscious, undisturbed normotensive rats failed to decrease arterial pressure (Fig 2), as has been reported previously. In rats made hypertensive by prolonged intravenous infusion of a low dose of Ang II, however, clonidine consistently reduced arterial pressure (Fig 1). The effect tended to increase in magnitude over the duration of the Ang II infusion period. Similar results were obtained in a study using the ganglionic blocker hexamethonium. The lack of HR changes after clonidine suggests that the antihypertensive effect was primarily due to a fall in vascular resistance. This finding supports earlier work indicating that low amounts of blood-borne Ang II can induce an increase in neurogenic vasoconstrictor activity, perhaps through actions on the area postrema. It also provides a basis for understanding the ability of clonidine to reverse renovascular hypertension without influencing plasma renin activity. Prolonged exposure to modest or occasional increments in plasma Ang II secondary to renal artery disease could gradually evoke an increase in sympathetic nervous activity—an increase suppressible by clonidine. In fact, elevated discharge frequency in muscle sympathetic nerves was recently documented in humans with renovascular hypertension. Angioplasty to relieve renal arterial stenosis decreased both sympathetic activity and arterial pressure.

What is the relative role of neurogenic pressor mechanisms in the slow pressor effect of blood-borne Ang II? Strong evidence suggests other important contributors. For example, alleviation of the fluid-retaining properties of Ang II prevents hypertension during chronic infusion of the peptide in dogs. Also, numerous experiments have highlighted the capacity of Ang II to bring about structural vascular modifications able to increase total peripheral resistance and arterial pressure. It is likely that these and perhaps other factors work in concert to produce the slow pressor effect. As just one possibility it should be noted that both volume retention and vascular hypertrophy would augment the vasoconstriction caused by even a normal level of sympathetic nervous activity.

The relative importance of the different elements of the slow pressor effect may vary among individuals and/or different durations of Ang II exposure.

In the current protocol, no evidence was found that Ang II significantly affected renal sodium and water excretion, consistent with previous similar experiments in rats. However, the animals studied here were already on a moderately high salt intake, which is known to facilitate the slow pressor effect of Ang II.
though clonidine can cause a modest natriuresis and diuresis in rats, this action did not appear to be of sufficient magnitude or duration in our study to influence arterial pressure.

Chronic infusion of Ang II has been shown to cause significant vascular hypertrophy in rats within 10 to 12 days, even when arterial pressure was prevented from increasing. Such an action may have been operative in raising arterial pressure in our study. But the observation that the hypertension produced by Ang II was rapidly attenuated by clonidine, and was fully reversed within 24 hours of terminating the infusion, argues against structural vascular change as the sole factor underlying the hypertension.

In summary, chronic intravenous infusion of a low dose of Ang II into rats caused a sustained hypertension that could be partially reversed by the centrally acting sympatholytic drug clonidine. Clonidine did not affect arterial pressure in normotensive rats. These results support previous studies indicating that the slow pressor effect of circulating Ang II is caused in part by neurogenic mechanisms.

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References

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