Effect of angiotensin II receptor blockade on autonomic nervous system function in patients with essential hypertension

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Krum, Henry, Elisabeth Lambert, Emma Windebank, Duncan J. Campbell, and Murray Esler. Effect of angiotensin II receptor blockade on autonomic nervous system function in patients with essential hypertension. Am J Physiol Heart Circ Physiol 290: H1706–H1712, 2006. First published November 11, 2005; doi:10.1152/ajpheart.00885.2005.—It has long been proposed that the renin-angiotensin system exerts a stimulatory influence on the sympathetic nervous system, including augmentation of central sympathetic outflow and presynaptic facilitation of norepinephrine release from sympathetic nerves. We tested this proposition in 19 patients with essential hypertension, evaluating whether the angiotensin receptor blockers (ARBs) eprosartan and losartan had identifiable antiadrenergic properties. This was done in a prospective, randomized, three-way placebo-controlled study of crossover design. Patients were randomized to 600 mg of eprosartan daily, 50 mg of losartan daily, or placebo. The treatment period was 4 wk, with 2-wk washout periods. Multiunit firing rates in efferent sympathetic nerves distributed to skeletal muscle vasculature (muscle sympathetic nerve activity, MSNA) were measured with microneurography, testing whether ARBs inhibit central sympathetic outflow. In parallel, isotope dilution methodology was used to measure whole body norepinephrine spillover to plasma. Mean blood pressure on placebo was 151/98 mmHg, with both ARBs causing reductions of ~11 mmHg systolic and 6 mmHg diastolic pressure, placebo corrected. Both MSNA [35 ± 12 bursts/min (mean ± SD) on placebo] and whole body norepinephrine spillover [366 ± 247 ng/min] were unchanged by ARB administration, indicating that the ARBs did not materially inhibit central sympathetic outflow or act presynaptically to reduce norepinephrine release at existing rates of nerve firing. These findings contrast with the easily demonstrable reduction in sympathetic nervous activity produced by antihypertensive drugs of the imidazoline-binding class, which are known to act within the brain to inhibit sympathetic nervous outflow. We conclude that sympathetic nervous inhibition is not a major component of the blood pressure-lowering action of ARBs in essential hypertension.

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INTERACTION BETWEEN the renin-angiotensin system and the sympathetic nervous system has traditionally been regarded to be bidirectional. The contribution of the renal sympathetic nerves to renal renin release, one component of this synergy, is explicit and well documented (23, 38), the juxtaglomerular apparatus receiving a rich postganglionic sympathetic innervation. The other component of this presumed angiotensin-sympathetic nervous synergy, facilitation of the sympathetic nervous system by angiotensin, also has a long research pedigree, dating back to the seminal observations of Dickinson (8) and Bickerton and Buckley (4), who observed that angiotensin injected into the dog carotid or vertebral artery activated the sympathetic nervous system. Angiotensin receptors and other components of the renin-angiotensin cascade are widely distributed in the brain (14). Over time, the idea that angiotensin exerts a stimulatory influence on the sympathetic nervous system has been extended to include almost all elements of the sympathetic neuraxis, including stimulation of central nervous system (CNS) sympathetic outflow, facilitation of ganglionic transmission, presynaptic regulation of neural norepinephrine release, and inhibition of neuronal norepinephrine reuptake (36).

We wondered whether this idea that angiotensin augments sympathetic nervous system function in such an all-encompassing fashion had been overstated. It is out of keeping with the typically absent or rather trifling stimulatory or antiadrenergic neural effects typically seen in humans when angiotensin is infused or the renin-angiotensin system is blocked by angiotensin-converting enzyme (ACE) inhibitors, respectively. There is, in fact, a striking mismatch between these absent or minimal sympathetic neural effects of angiotensin administration and angiotensin blockade in humans (1, 16, 17, 25, 43) and the prominent sympathetic neural stimulation produced by angiotensin in laboratory experiments (2, 6, 10, 27, 42).

One concept of particular clinical interest is the claim that angiotensin receptor blocking agents (ARBs) may, by blocking the presynaptic ANG II type 1 (AT1) receptor, reduce ANG II-mediated facilitation of neurally sustained vascular tone, contributing to the antihypertensive action (3, 33). Although in most studies in humans angiotensin administration has not been observed to increase central sympathetic outflow (16), a small but measurable presynaptic stimulatory neural action of angiotensin has been unequivocally demonstrated in the sympathetic nerves of the forearm (5). The ARB eprosartan has been observed in preclinical studies to be a potent inhibitor of neural presynaptic AT1 receptors (3, 33). It has been postulated that this effect may be greater than that observed with other ARBs at pharmacologically relevant doses. This hypothesis has not been formally tested in humans.

In the present study we tested whether two ARBs, eprosartan and losartan, possess presynaptic antiadrenergic activity in patients with essential hypertension, making comparisons with placebo. We measured multiunit firing rates in postganglionic efferent sympathetic nerve fibers distributed to the skeletal

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muscle vasculature with microneurography, to test whether ARBs inhibit central sympathetic outflow. In parallel, we used isotope dilution methodology to measure whole body norepinephrine spillover to plasma. Our proposition was that reduction in norepinephrine spillover rates with ARBs disproportionate to any reduction in sympathetic nerve firing rates would most likely represent a presynaptic, antiadrenergic action of the drug class.

METHODS

Study Design

This was a prospective, randomized, three-way placebo-controlled crossover study design. Patients were randomized to 600 mg of eprosartan each morning, 50 mg of losartan each morning, or placebo. The treatment period was 4 wk, with a 2-wk washout period between each treatment period. We considered 2 wk an adequate washout period based on the half-life of the active therapies. Indeed, many crossover studies of ARBs do not include a washout period at all (24, 37). Autonomic assessment and measurement of plasma ANG II concentration were done at the end of each 4-wk treatment period. Measurements were made at peak plasma drug concentrations at ~2 h after oral dosing.

Inclusion criteria were age of 18–70 yr, a body mass index (BMI) of 18–35 kg/m², and either newly diagnosed mild to moderate essential hypertension or the ability to be safely withdrawn from antihypertensive medication. At the end of a 2- to 4-wk screening period, for inclusion patients needed to have a sitting diastolic blood pressure (mean of 3 readings, measured by manual sphygmomanometer) of 90–105 mmHg and systolic blood pressure >140 mmHg. Patients with any comorbidity that mandated the use of a specific antihypertensive drug class were excluded. Patients were not permitted to receive any other medication that could influence autonomic activity. The study was approved by the Alfred Hospital Human Ethics Committee (Study No. 64, year 2000), and written informed consent was obtained from all patients.

Assessment of Autonomic Nervous System

In addition to blood pressure and heart rate recording, measurement of specific aspects of sympathetic and parasympathetic nervous system function, involving neurochemical and electrophysiological measurement of sympathetic nervous activity, monitoring of heart rate variability (HRV), and testing of reflex sympathetic responses were performed. These evaluations were done over two consecutive study days, in the order described below.

Isotope Dilution Determination of Norepinephrine and Epinephrine Plasma Kinetics

On attendance of the subjects at the research laboratory on the morning of the first study day, a 21-gauge plastic cannula was inserted percutaneously under local anesthesia without cutdown in a radial or brachial artery for direct measurement of intra-arterial pressure and for blood sampling. A peripheral venous line was placed for infusion of radiolabeled norepinephrine and epinephrine, allowing measurement of catecholamine plasma kinetics by isotope dilution, utilizing the arterial plasma samples (11). Determination of endogenous and radiolabeled forms of norepinephrine and epinephrine was by HPLC and chromatogram peak fraction collection (29). The appearance rate of epinephrine in plasma represented adrenal medullary secretion. The total rate of spillover of the sympathetic neurotransmitter norepinephrine to plasma was used as an index of whole body sympathetic nerve firing rates, subject to any influence of presynaptic modification of norepinephrine release and alteration in neuronal norepinephrine re-uptake (11).

Measurement of Reflex Sympathetic Nervous Responses

Maximum blood pressure and heart rate change with the cold pressor test was used as a measure of sympathetic cardiovascular response (9). To perform this test, patients were asked to insert their arm (other than the one studied for norepinephrine kinetics) into a water bath, filled with ice and water, for 2 min. The plasma concentration of norepinephrine was also measured during head-up tilt.

Sympathetic Nerve Recording by Microneurography

On the second study day, microneurography was used to record multiunit postganglionic sympathetic activity in a muscle fascicle of the peroneal nerve at the fibular head (40, 41). The common peroneal nerve was located by palpation and electrically stimulated via a surface probe. A tungsten microelectrode (FHC, Bowdoinham, ME) was then inserted percutaneously and adjusted until satisfactory spontaneous muscle sympathetic nerve activity (MSNA) was observed in accordance with previously described criteria (40, 41). Resting measurements were performed over a 15-min period.

Cardiac Vagal

Parasympathetic nervous system activity was assessed with measures of HRV, as previously described in detail by our group (35). Measurements were made from 24-h Holter monitoring, commenced on the first study day.

Presynaptic Neuromodulation of Norepinephrine Release

We tested for reduction in norepinephrine spillover rates with ARBs disproportionate to any possible reduction in sympathetic nerve firing rates produced by inhibition of central sympathetic outflow. A selective or disproportionate reduction in norepinephrine release to plasma, beyond that expected from any reduction in sympathetic nerve firing rates, would most likely signify a presynaptic, antiadrenergic action of the ARB.

Measurement of Plasma Angiotensin Concentration

Plasma ANG II levels in the supine position were measured after 30 min of supine rest. After collection, arterial blood (10 ml) was immediately added to chilled, heparinized tubes (in an ice bucket) containing 0.5 ml of an enzyme inhibitor cocktail [125 mmol/L EDTA, 2 mmol/L CP-38560A (renin inhibitor), 50 mmol/L 1,10-phenanthroline, 1 g/L neomycin sulfate, and 2% ethanol in water], mixed, and immediately centrifuged at 4°C for 10 min. The plasma was snap frozen on dry ice and stored at −80°C until being extracted with C18 Sep-Pak cartridges and assayed for ANG II with HPLC-based radioimmunoassay (26).

Statistical Analysis

A sample size of 19 patients who could be evaluated had 90% power to detect a mean difference of 20% in the study primary end point (plasma norepinephrine spillover rate) between eprosartan and placebo, using a level of significance of 0.05 in two-sided testing and a Bonferroni adjustment for the two comparisons of interest. This assumed a standard deviation of 20% in the primary end point. Comparisons between treatment groups were made by analysis of variance with pairwise comparison of eprosartan vs. losartan and eprosartan vs. placebo by Student’s unpaired t-test. Comparisons at baseline and end of study phase (e.g., for blood pressure and heart rate) were made by Student’s paired t-test. All values are shown as means ± SD unless otherwise stated.

RESULTS

Baseline Characteristics

Baseline demographic and laboratory parameters are summarized in Table 1. Mean blood pressure was 151/98 mmHg,
Blood Pressure and Heart Rate

Eprosartan and losartan caused an ~11-mmHg fall in systolic blood pressure and a 6-mmHg fall in diastolic blood pressure, placebo corrected (Table 2). Changes in blood pressure were statistically significant for the active treatments. Heart rate (placebo corrected) while seated did not differ between groups.

Plasma Concentration of ANG II

Plasma ANG II concentration after placebo was 3.1 fmol/ml (geometric mean; 95% confidence interval 1.0, 9.5). There was an increase in the plasma concentration of angiotensin after both eprosartan [to 6.5 fmol/ml (2.0, 21.5); P < 0.01] and losartan [to 7.6 fmol/ml (1.3, 44.5)] treatment.

Measures of Sympathetic Nervous Activity

Norepinephrine plasma kinetics. The study primary end point, plasma norepinephrine spillover rate at rest, was not significantly different across the three groups, although it was marginally lower in the eprosartan group (Table 3; Fig. 1). The effect of ARB on plasma norepinephrine spillover rate was evaluated according to resting plasma ANG II concentration in an exploratory analysis.

In patients with low resting plasma ANG II concentrations (below the median), plasma norepinephrine spillover rate was 258.3 ± 120.7 (placebo), 307.1 ± 113.9 (losartan), and 293.6 ± 118.1 (eprosartan) ng/min (P = not significant [NS] between groups). Patients with high resting plasma ANG II concentrations (above the median) had a plasma norepinephrine spillover rate of 462.8 ± 294.3 (placebo), 430.3 ± 193.5 (losartan), and 337.8 ± 171.4 (eprosartan) ng/min (P = NS between groups). In comparison with placebo, whole body norepinephrine spillover on eprosartan was 13.4% lower (95% confidence interval 100.2, 101.8%).

Measurement of reflex sympathetic nervous responses. There were no significant changes in baseline plasma norepinephrine concentration (Table 3) or in plasma norepinephrine responses to upright tilt across the three treatment groups.
Similarly, there were no ARB drug effects on the cold pressor test, assessed either by systolic or diastolic blood pressure or by heart rate responses (Table 3).

Sympathetic nerve recording by microneurography. Multi-unit postganglionic nerve firing rates in sympathetic efferents directed to the skeletal muscle vasculature were similar across the three treatment groups (Table 3; Fig. 3), arguing against inhibition of central sympathetic outflow by the ARBs.

Cardiac Vagus

In the time domain, there was no significant difference in mean R-R interval, SD of normal R-R intervals, percentage of intervals that varied >50 ms from the previous interval, or root mean square of successive normal R-R interval differences across the three treatment groups (Table 3). Similarly, in the frequency domain, heart rate total spectral power and high-frequency spectral power were similar across the three groups (Table 3).

Presynaptic Neuromodulation of Norepinephrine Release

The absence of any reduction in whole body norepinephrine spillover at sympathetic nerve firing rates that were unchanged suggested that neither ARB exerted a presynaptic action on norepinephrine release from sympathetic nerves.

DISCUSSION

A large body of preclinical research suggests that the renin-angiotensin system facilitates the sympathetic nervous system and that ACE inhibition and angiotensin receptor blockade are antiadrenergic (2, 3, 6, 10, 27, 33, 42). At a time when inhibition of the sympathetic nervous system has come to be seen as beneficial, most evident in the use of β-adrenoceptor blockers in heart failure (34), pharmaceutical industry marketing strategies have certainly helped perpetuate the notion that these drug classes rank as antiadrenergic agents on an equal footing with β-adrenergic blockers and centrally acting sympathetic suppressants.

Evidence in humans that angiotensin augments the sympathetic nervous system is much weaker, or absent (1, 16, 17, 25, 43). The present study sought to test this interrelationship in humans, utilizing ARBs in a clinically relevant population, patients with essential hypertension. Because sympathetic nervous system activity is commonly increased in patients with essential hypertension and appears to be involved in the initiation and maintenance of the blood pressure elevation (13), a reduction in sympathetic activity by ARBs should be clinically beneficial. We measured multiunit firing rates in postganglionic efferent sympathetic nerve fibers distributed to the skeletal muscle vasculature with microneurography to test whether ARBs inhibit central sympathetic outflow. In parallel, we used isotope dilution methodology to measure whole body norepinephrine spillover to plasma. A reduction in norepinephrine spillover rates with ARBs disproportionate to any reduction in sympathetic nerve firing rates should signify a presynaptic, antiadrenergic action of the drug class. The caveat, however, does apply here that sympathetic nerve firing and norepinephrine release were not measured in the same sympathetic outflow, nerve firing being that in the sympathetic efferents to the
skeletal muscle vasculature while norepinephrine release was for the whole body. A regional differentiation of drug sympathetic responses could undermine this method of testing for an ARB presynaptic action on sympathetic nerves.

The trial was of rigorous design, using a three-way, cross-over, chronic dosing configuration, with appropriate washout periods and measurement of autonomic effects during peak drug actions at maximal concentration after dosing. The expected reductions in blood pressure were observed with both ARBs, confirming the clinical efficacy of these agents in lowering blood pressure at these doses. Both drugs also increased plasma ANG II concentrations, as would be expected with an AT\textsubscript{1} receptor blocker. The similar increases in ANG II levels with eprosartan and losartan indicate that the two doses were relatively well matched with respect to AT\textsubscript{1} receptor blockade.

Multunit postganglionic nerve firing rates in sympathetic efferents directed to the skeletal muscle vasculature were similar across the three treatment groups. Whole body norepinephrine spillover was unchanged by ARB administration. Similarly, plasma norepinephrine concentrations in the supine position and during head-up tilting and responses to the cold pressor test were not reduced by eprosartan or losartan. These findings indicate that the ARBs did not materially inhibit central sympathetic outflow or act presynaptically to reduce sympathetic neural norepinephrine release. The observation that blood pressure was significantly lowered by these agents at the doses utilized suggests that sympathetic nervous inhibition makes minimal contribution to blood pressure lowering by ARBs in essential hypertension. These findings with eprosartan and losartan contrast with the pronounced, easily demonstrable, reduction in norepinephrine spillover and sympathetic nervous activity produced by antihypertensive drugs of the imidazoline-binding class, such as moxonidine and rilmenidine, which act within the brain to inhibit sympathetic nervous outflows (12). These contrasting findings are of some relevance as eprosartan crosses the blood-brain barrier and has been found to exert central inhibitory effects on AT\textsubscript{1} receptors (31).

That having been said, it is worth noting that similar if not lower levels of sympathetic activity were observed with both ARBs vs. placebo, despite lower achieved blood pressure levels. In this context, it might be said that we have not adequately considered that during the periods of angiotensin blocker treatment, when blood pressure had been lowered, arterial baroreflex drive would have tended to elevate sympathetic nerve firing rates and norepinephrine spillover and, furthermore, that the unchanged sympathetic activity we found during ARB administration can really arise only if the drug class has sympathetic inhibitory properties. This perhaps could be tested for in two ways, by measuring sympathetic activity at a time when the blood pressure had been acutely restored in some way to the pretreatment levels and by performing a full sympathetic-arterial baroreflex curve and noting what sympathetic nerve firing rates were at the blood pressure point on the curve that corresponded to the pretreatment pressure. This line of thinking might be extended to suggest that although the centrally acting imidazoline-binding drugs, such as rilmenidine and moxonidine, do lower sympathetic activity materially, the direct comparison we make with ARBs may not be valid because of differing influences of the two drug classes on the baroreflex.

Although there is merit to an argument of this type, in our view this particular line of reasoning does not give sufficient weight to the very real probability that with over 1 mln of blood pressure reduction on the ARBs, there will have been a resetting (lowering) of the operating point for the baroreflex. If such resetting were to have occurred, to test sympathetic activity during ARB treatment at a time when the blood pressure is acutely elevated back to baseline would introduce an artifact and could lead to misinterpretation.

The findings of the present study with ARBs are similar to those from an unblinded study with an ACE inhibitor (lisinopril) in patients with essential hypertension, done by Grassi and colleagues (17), who found no reduction in MSNA. In a previously reported study in healthy volunteers, no reduction in muscle sympathetic activity was seen with eprosartan administration (MSNA actually increased significantly; Ref. 19). Here, shorter-term drug dosing than what we applied was used, the observed sympathetic activation perhaps being attributable to activation of the arterial baroreflex during the early phase of blood pressure lowering. For this reason, as indicated above, longer-term studies (such as our own) are required to determine the true autonomic modulatory effects of a chronic therapy such as the ARBs.

Cardiac vagal withdrawal is present in patients with essential hypertension, which is possibly of clinical relevance given that low parasympathetic tone in the heart has been associated with adverse clinical cardiovascular outcomes (21). As for the sympathetic nervous system, however, we found little evidence that cardiac vagal tone was modified by the ARBs.

As we failed to detect any reduction in central sympathetic outflow or presynaptic inhibition of norepinephrine release by ARBs in patients with essential hypertension, this contrasts with many previous reports that suggest that angiotensin augments sympathetic nervous activity at multiple levels of the sympathetic neuraxis (23, 38), we now consider the possible basis for these discrepancies.

**Differing Findings with Administration of Angiotensin vs. Angiotensin Blockade?**

Evidence supporting a stimulatory influence of angiotensin on the sympathetic nervous system has more commonly been drawn from studies involving the administration of angiotensin than its blockade. In laboratory experiments, the doses and concentrations of angiotensin used have often been very high (6, 15, 27, 30, 42), sometimes clearly supraphysiological. The difficulty of judging the appropriateness of an administered dose applies particularly in the case of CNS administration of angiotensin (2). In general, the results obtained through blockade of the renin-angiotensin system, assessing a sympathetic influence by the measured subtraction of sympathetic activity achieved, are probably more trustworthy than those from experiments in which angiotensin is administered, often in inappropriately high doses.

**Special Characteristics of Studies in Humans**

There are, in general, small or no antiadrenergic effects observed with blockade of the renin-angiotensin system by ACE inhibitors and ARBs in humans (1, 17, 25, 43), results that are strikingly discordant from those obtained in many laboratory experiments (2, 6, 10, 27, 42). This is not due, as has
been claimed, to the insensitivity of the available methods for studying sympathetic nervous function clinically. Contemporar-ary methods for measuring human sympathetic nerve firing by microneurography (40, 41) and rates of norepinephrine release from sympathetic nerves by isotope dilution (11) are both sensitive and valid and not inferior to the methodology available in the experimental animal laboratory. The contrasting results appear to derive from differences in study design, such as the avoidance in humans on the grounds of safety and ethics of peripheral administration of angiotensin in supraphysiologi- cal doses and of CNS administration in any dose, and to a preference for study designs involving renin-angiotensin blockade rather than angiotensin administration (1, 17, 25, 43).

Is Sympathetic Inhibition by Renin-Angiotensin Blockade in Cardiac Failure a Special Case?

In heart failure, the claim that there is a direct facilitatory influence of angiotensin on the sympathetic nervous system and that this is specifically blocked by antagonism of the renin-angiotensin system (7, 28, 32) appears strong on face value. In this context, it is of interest that the greatest reductions (albeit nonsignificant) in sympathetic activity in the present study with eprosartan administration to hypertensive patients (at least for the primary study end point of plasma norepinephrine spillover rate) occurred in those with the highest baseline levels of ANG II in plasma. The widely held view that renin-angiotensin antagonism is sympathoinhibitory may, however, be a misconception based on two sources of error. The first of these is the misinterpretation of the significance of changing plasma norepinephrine values under therapy. The plasma concentration of norepinephrine, commonly used as a sympathetic index in clinical studies, typically falls during the course of treatment of cardiac failure with ACE inhibitors, but this is due in part to hemodynamic improvement increasing regional blood flows and the plasma clearance of norepinephrine (11). The second basis for misconception is that sympathethetic nervous activity falls with effective treatment in the heart failure patient whatever drugs are used. Sympathetic tone in the failing human heart is approximately three times higher in minimally treated patients with New York Heart Association class III and IV failure than in optimally treated patients of the same heart failure severity (18, 22), independent of whether the antifailure drugs used antagonize the renin-angiotensin system or not.

Clinical Perspectives

Activation of the sympathetic nervous system appears to be important in patients with essential hypertension at several levels, commonly initiating and sustaining the blood pressure elevation (13), contributing directly to the development of left ventricular hypertrophy (39), and causing insulin resistance, through neurogenic vasoconstriction in skeletal muscle lowering blood flow and consequently reducing muscle glucose delivery and uptake (20). Because of the longstanding viewpoint that the renin-angiotensin system exerts an important regulatory influence over the sympathetic nervous system, drugs antagonizing the renin-angiotensin system, ACE inhibitors and ARBs, have been thought to inhibit the sympathetic nervous system and be well placed to reverse the neural pathophysiology of essential hypertension. We found that of two ARBs tested in patients with essential hypertension, epro-sartan and losartan, neither exerted an antiadrenergic effect. If antihypertensive drug therapy is to be dictated by considerations of the underlying pathophysiological mechanisms of hypertension, and this of course can only be one consideration on which therapy choices are made, reversal of sympathetic activation in hypertension cannot be expected with renin-angiotensin antagonism. Drugs with specific sympathetic antagonism, such as the centrally acting imidazoline-binding drugs moxonidine and rilmenidine, will be required.

REFERENCES