

Elevation of Sympathetic Activity by Eprosartan in Young Male Subjects

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Background: Selective blockade of the type 1 angiotensin II receptors (AT1 receptors) reduced blood pressure (BP) elevation caused by sympathetic stimulation in the pithed rat model. This has been attributed to blockade of AT1 receptors located presynaptically on sympathetic nerve endings normally facilitating norepinephrine release. We examined the effects of AT1 receptor blockade on the sympathetic nervous system in humans.

Methods: Twenty-nine young white men with normal to mildly hypertensive BP values participated in a double-blind, placebo-controlled, randomized cross-over protocol receiving 600 mg/d of eprosartan or placebo for 1 week. At the last day of intake we measured hemodynamic parameters, muscle sympathetic nerve activity by microneurography, and plasma levels of norepinephrine, epinephrine, and angiotensin II during rest and cardiovascular stress.

Results: Eprosartan lowered resting mean arterial pressure (73.6 ± 11.0 v 78.0 ± 10.3 mm Hg, $P < .05$; Finapres, Ohmeda, Englewood, CO), and elevated heart rate (64.4 ± 7.6 v 61.1 ± 6.8 beats/min, $P = .01$), muscle sympathetic nerve activity (14.1 ± 10.4 v 9.8 ± 6.3

bursts/min, $P < .05$) and plasma angiotensin II (37.0 ± 33.7 v 6.9 ± 2.8 ng/L, $P < .01$), as well as norepinephrine levels (234.2 ± 87.6 v 187.8 ± 59.3 ng/L, $P < .01$). Eprosartan did not blunt sympathetic activation caused by lower body negative pressure or mental stress.

Conclusions: These results contrast with animal data showing antiadrenergic properties of this drug. If any, it appeared, that eprosartan causes augmented central neural vasoconstrictor outflow paralleled by increased plasma levels of norepinephrine, which casts doubt on its ability to dampen norepinephrine release from peripheral sympathetic nerve endings in humans. We hypothesize that eprosartan leads to a resetting of the baroreflex, presumably by the markedly elevated circulating angiotensin II. Am J Hypertens 2003;16:658–664 © 2003 American Journal of Hypertension, Ltd.

Key Words: Clinical studies, cardiovascular pharmacology, angiotensin converting enzyme inhibitors/angiotensin receptors, autonomic, reflex, and neurohumoral control of the circulation.

Blockade of the renin-angiotensin system (RAS) by angiotensin converting enzyme (ACE) inhibitors has been demonstrated to be beneficial in the therapy of essential hypertension, heart failure, and postmyocardial infarction. It has been found that they inhibit not only the RAS, but also the sympathetic nervous system (SNS) in both, healthy volunteers¹ and in patients characterized by an activated SNS (eg, in heart failure^{2,3} and in renal failure⁴). It is widely assumed that antagonizing the angiotensin II (Ang II) receptor subtype 1 (AT1 receptor) influences the SNS in a comparable manner. In fact, using the pithed rat model, it has been shown that the blood pressure (BP) elevation elicited by

artificially generated sympathetic outflow may be blunted by various AT1 blockers.^{5–8} The causal inhibition of the increase in vascular resistance has been attributed to a presynaptic mechanism. Angiotensin II facilitates norepinephrine release from sympathetic terminals by binding to presynaptic AT1 receptors. Blocking these receptors might lead to diminished norepinephrine release and therefore lesser vasoconstriction. It has been demonstrated in rats that candesartan inhibits Ang II-mediated catecholamine release from sympathetic nerve endings as well as the adrenal medulla through inhibition at the specific AT1 receptor site.⁹ Furthermore, the importance of presynaptic rather than postsynaptic AT1 re-

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ceptors has been confirmed by the finding that α -adrenoceptor-mediated BP elevation by exogenous norepinephrine was not affected by AT1 blockade.⁵

Although the studies in rats corroborated the interconnection of the RAS and the SNS in the periphery, they did not answer the question whether sartanes are also able to dampen central sympathetic outflow. Furthermore, the findings obtained in rats may not translate directly to human beings. Struck et al¹⁰ addressed this issue and could not find valsartan to inhibit central sympathetic outflow in hypertensive subjects. Furthermore, the results of the Evaluation of Losartan In The Elderly (ELITE) study supposed superiority of losartan over ACE inhibition by captopril in elder heart failure patients,¹¹ but later studies—ELITE-II¹² and Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD)¹³—could not confirm this finding.

It is difficult to infer direct drug effects on the autonomic nervous system from studies in patients with cardiovascular disease because improvement of the hemodynamic state causes secondary amelioration in autonomic function. Thus, to reveal drug interactions with regulatory systems it is reasonable to do so under physiologic conditions. Therefore, this study in humans devoid of more severe forms of cardiovascular disease seeks to solve the following two questions: Is the AT1 blocker eprosartan able to dampen central sympathetic outflow to the vasculature or the peripheral release of catecholamines? Does eprosartan have the potency to blunt provoked sympathetic activation as has been shown in animal models?

Methods

Study Population

We recruited 29 young white men in this prospective, double-blind, randomized, placebo-controlled, crossover study. They were screened by history, physical examination, and routine blood tests to exclude moderate, severe,¹⁴ or secondary forms of hypertension, organic diseases, confounding medication, and alcohol or drug abuse. According to 24-h ambulatory BP monitoring their arterial pressures were below 160/100 mm Hg. The participants were on no special diet (ie, without salt restriction). Informed written consent was obtained before the study and the protocol was approved by the Ethical Committee of the University of Erlangen-Nuremberg.

Measurements

Hemodynamic Parameters Twenty-four-hour ambulatory BP profile was achieved by using an automated portable device (Spacelabs Medical, Redmond, WA). Beat-to-beat arterial pressure was taken noninvasively by the photoplethysmographic finger device Finapres (Ohmeda, Englewood, CO). Central venous pressure (CVP) was measured by a 16-gauge indwelling catheter inserted

through an antecubital vein and advanced to the superior vena cava.

Heart rates were counted from the electrocardiogram. Forearm vascular resistance was calculated from arterial BP (Finapres) and forearm blood flow, the latter being gained by venous occlusion plethysmography by means of a mercury-in-Silastic strain gauge at the left forearm.¹⁵ Because of the calibration of the strain gauge, absolute values of forearm vascular resistance were obtained. For brevity we use “unit” instead of “mm Hg/(mL/min/100 mL).”

Muscle Sympathetic Nerve Activity To measure sympathetic activity we used the technique of microneurography introduced by Vallbo and Hagbarth in 1967¹⁶ and extensively reviewed later.¹⁷ Herewith, we obtained multiunit recordings of postganglionic sympathetic nerve activity with unipolar tungsten microelectrodes inserted selectively into muscle nerve fascicles of the right peroneal nerve posterior to the fibular head. For quantification, sympathetic bursts were identified by inspection of the mean voltage neurograms depicting characteristic bursts of C-fiber action potentials as upward peaks. Recordings were considered suitable for analysis if the ratio between the amplitude of the largest bursts and baseline noise was at least 3:1. The rate of sympathetic nerve discharges was expressed as the number of bursts per minute (burst frequency) and—corrected for heart rate—as bursts per 100 heart beats (burst incidence). All nerve recordings were analyzed by two investigators who were unaware of the subjects’ medication. Presented data mirror their common decisions.

Neurohormonal Substances Blood samples were collected from an indwelling venous cannula at the right forearm into cooled tubes containing EDTA (for Ang II and catecholamines) and glutathione (for catecholamines). Plasma was stored at -21°C (Ang II) or -70°C (catecholamines) until completion of the study and then analyzed. Plasma Ang II levels were determined by radioimmunoassay as previously described,¹⁸ and plasma catecholamines (epinephrine and norepinephrine) by high-performance liquid chromatography (HPLC) according to Raasch et al.¹⁹

Interventions

To obtain conditions of elevated sympathetic activity, we performed two different cardiovascular stress tests. The first one consists of 30 min of lower body negative pressure (LBNP) of -15 mm Hg. This suction was applied using an air tight chamber surrounding the lower half of the subject’s body beginning at the level of the iliac crest. This maneuver causes unloading of cardiopulmonary receptors leading to an elevation of vasoconstrictor nerve traffic, norepinephrine plasma levels, and vascular resistance.

The second test, mental stress, was imposed by means

Table 1. Subjects' characteristics and resting values (rest 1) of chosen variables during placebo and eprosartan

Parameter	Placebo	Eprosartan	n	P
Age (y)	26.7 ± 3.9		27	
BMI (kg/m ²)	23.5 ± 2.3		27	
SAP ₂₄ (mm Hg)	127.0 ± 9.1	124.1 ± 7.8	27	<.05
DAP ₂₄ (mm Hg)	76.0 ± 6.9	73.3 ± 7.1	27	<.01
MAP ₂₄ (mm Hg)	92.2 ± 6.4	89.6 ± 6.4	27	<.01
SAP _{fin} (mm Hg)	117.3 ± 15.5	111.2 ± 14.2	26	<.05
DAP _{fin} (mm Hg)	61.5 ± 9.1	58.2 ± 10.3	26	.07
MAP _{fin} (mm Hg)	78.0 ± 10.3	73.6 ± 11.0	26	<.05
FVR (u)	10.0 ± 4.0	8.5 ± 4.1	25	.08
CVP (mm Hg)	7.3 ± 1.1	6.6 ± 1.8	24	.10
HR (beats/min)	61.1 ± 6.8	64.4 ± 7.6	27	<.05
MSNA _f (bursts/min)	9.8 ± 6.3	14.1 ± 10.4	22	<.05
MSNA _i (bursts/100 hb)	16.3 ± 10.3	22.2 ± 15.1	22	<.05
Ang II (ng/L)	6.9 ± 2.8	37.0 ± 33.7	27	<.01
NE (ng/L)	187.8 ± 59.3	234.2 ± 87.6	26	<.01
E (ng/L)	44.3 ± 21.7	45.4 ± 20.5	27	ns

BMI = body mass index; SAP/DAP/MAP = systolic/diastolic/mean arterial pressure (24 h, Finapres); FVR = forearm vascular resistance (u = mL/min/100 mL forearm tissue); CVP = central venous pressure; HR = heart rate; MSNA_f/i = muscle sympathetic nerve activity (frequency [bursts/min], incidence [bursts/100 heart beats]); Ang II = plasma angiotensin II; NE = norepinephrine; E = epinephrine; ns = not significant.

Values are means ± SD.

of a computer game specially written for that purpose. Subjects had to move a racket with their right thumb (with a trackball) to hit a flying ball. The aim of the game is to destroy a wall brick-by-brick with the help of the ball. Once a wall is completely destroyed a new one appears. This repeats as long as the predefined time of 30 min is elapsed. (Thus, this game is a variant of blockout/breakout, which is well-known in the computer game community.) To ensure maximal effort, 1) different skills of the subjects were detected by the program itself resulting in becoming slower or faster continually, and 2) the subjects were paid according to the reached score.

Protocol

The participants of the study (for characteristics, see Table 1) were randomly assigned to receive placebo or eprosartan (600 mg/d) first for 7 days (intake phase 1). After a wash-out period of at least 4 weeks subjects started to take the other drug (cross-over, intake phase 2). On days 1 to 6 subjects were required to take their medication at 9 AM. On day 6 of intake of the study medication 24-h ambulatory BP monitoring was initiated. On day 7 subjects had a light meal at noon and their medication at 2 PM in the laboratory.

To guarantee a constant delay between the last intake and the next measurements, as well as comparable temporal conditions between phase 1 and phase 2 (placebo versus eprosartan), we followed a strict timetable as shown in Fig. 1. Within the first period of 90 min we prepared the subject, including a comfortable recumbent position in the suction chamber, inserted the central venous catheter and peripheral venous cannula, connected measurement devices, and searched for a suitable needle position in the nerve. Because the latter procedure can be time consum-

ing, we reserved approximately 60 min. In most cases, however, we completed the search earlier, resulting in a waiting period for the remaining minutes. Within this period maximal plasma concentrations of eprosartan (in case of real drug) are achieved.

Thereafter, the four measurement sections started: Rest 1, LBNP, Rest 2, and Mental Stress. As shown for LBNP in the lower part of Fig. 1, each section lasted 30 min. We allowed variables to reach steady state within 20 min before the actual data acquisition of 6 min duration. Immediately before data acquisition, the Finapres device was allowed to perform calibration. Immediately afterwards, blood samples were drawn.

Subjects were examined in supine position throughout the session.

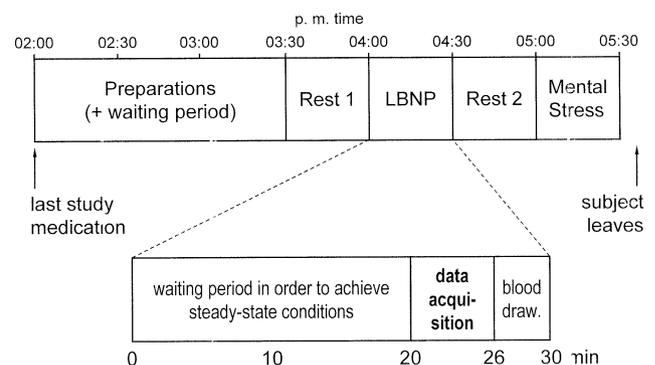


FIG. 1. Session timetable. The magnification below shows one of the measurement sections of 30 min duration (lower body negative pressure [LBNP], in this case) in detail. This scheme (20 + 6 + 4 min) is applied to the other sections as well, ie, to Rest 1, Rest 2, and Mental Stress.

Statistics

Two of the 29 enrolled volunteers did not complete the study. Therefore, only 27 data pairs could be included in statistical evaluation. The number of available data pairs for comparison of means is given in the column “*n*” of the tables (eg, muscle sympathetic nerve activity [MSNA], being the parameter that is most difficult to obtain, yielded only 22 pairs as shown in Table 1). Norepinephrine data of one subject during the placebo phase were more than twice as high as the maximum values of the remaining participants (644 ng/L). Moreover, with eprosartan, this parameter was comparable to that of the other subjects. According to Dixon’s Q-test we rejected his norepinephrine data from statistical analysis.

All values are given as mean \pm SD. Responses to cardiovascular stress are represented by the differences between the stress values and the preceding resting period (ie, LBNP – Rest 1 and Mental Stress – Rest 2). Two-tailed Student *t* test for paired data was used to compare parameter values of the eprosartan versus placebo session, or of stress versus resting baseline. Linear association between variables was assessed by correlation analysis. A value of $P < .05$ was considered statistically significant.

Results

Comparison of Resting Variables

Hemodynamic Parameters As expected, eprosartan reduced arterial BP (see Table 1). This is true for total 24-h, day, and nighttime measurements as well. During recumbent rest in our laboratory (Rest 1) Finapres values confirmed this reduction.

Forearm vascular resistance and central venous pressure tended to decline with eprosartan, whereas heart rate was increased.

During section Rest 2 arterial BP (Finapres), heart rate, and central venous pressure during eprosartan were not significantly different from placebo (data not shown).

MSNA Frequency and (heart rate corrected) incidence of sympathetic bursts was significantly increased during the eprosartan Rest 1 section as compared to the placebo phase (the representative original recordings are shown in Fig. 2A and bar graphs show group values in Fig. 2B). This increase was even more pronounced during section Rest 2 (10.5 ± 8.4 v 16.3 ± 10.0 bursts/min, $P < .01$; 17.1 ± 14.0 v 25.6 ± 14.5 bursts/100 heart beats, $P < .01$).

Neurohormonal Substances Eprosartan led to increases in plasma Ang II levels by more than fivefold. Norepinephrine levels increased significantly (Fig. 2B), whereas epinephrine was not influenced by eprosartan. The comparison of these parameters during section Rest 2 shows similar results (data not shown).

There was no significant correlation between eprosartan-induced changes in plasma Ang II and norepinephrine

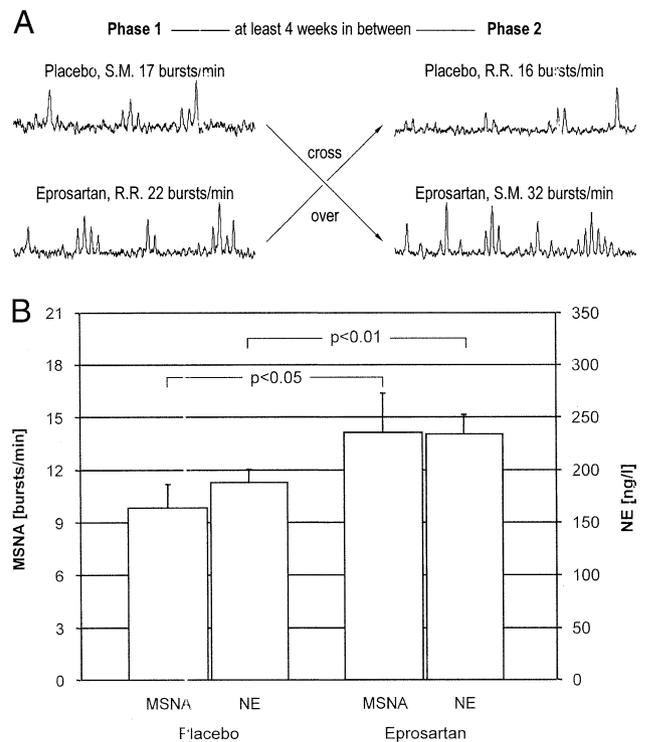


FIG. 2. A) Representative original recordings of resting muscle sympathetic nerve activity at the end of a 1-week administration of placebo and eprosartan in the same two subjects (S.M., R.R.). These recordings were obtained during section Rest 1 (see protocol). The traces of 30 sec duration show an increase of muscle sympathetic nerve activity (MSNA) from placebo to eprosartan session in both subjects. B) Resting MSNA and plasma norepinephrine (NE) levels during the placebo versus eprosartan session. Bars represent means \pm SE.

levels ($r = 0.075$, $P =$ not significant [ns]) or MSNA ($r = -0.127$, $P =$ ns).

Comparison of Responses to Cardiovascular Stress

As intended, LBNP significantly reduced central venous pressure in either phase (see Table 2). The obtained increases of MSNA, Ang II, and norepinephrine, however, were not different.

As expected, arterial BP and heart rate were markedly elevated by mental stress. Central venous pressure and forearm vascular resistance were significantly reduced. The MSNA, on the other hand, was not altered. The increase of Ang II was small but significant. Mental stress elevated epinephrine plasma levels significantly but only tended to do so with norepinephrine (data not shown). Eprosartan did not change the responses to mental stress except for a smaller increase in heart rate.

Discussion

Main Results

Unexpectedly, 1-week administration of the AT1-receptor antagonist eprosartan at a daily dosage of 600 mg/d sig-

Table 2. Comparison of responses to cardiovascular stress

Parameter	Lower Body Negative Pressure			Mental Stress		
	Placebo	Eprosartan	n	Placebo	Eprosartan	n
Δ SAP _{fin} (mm Hg)	+3 ± 8	+4 ± 11	25	+13 ± 12	+9 ± 14	24
Δ DAP _{fin} (mm Hg)	+4 ± 5	+5 ± 7	25	+10 ± 8	+8 ± 9	24
Δ MAP _{fin} (mm Hg)	+4 ± 5	+4 ± 8	25	+12 ± 9	+10 ± 9	24
Δ FVR (u)	+3.4 ± 2.8	+4.2 ± 4.1	24	-2.3 ± 2.9	-2.5 ± 3.3	24
Δ CVP (mm Hg)	-4.4 ± 1.0	-3.8† ± 1.2	23	-1.0 ± 1.2	-0.9 ± 0.8	21
Δ HR (beats/min)	+2 ± 4	+2 ± 3	26	+15 ± 9	+12* ± 7	25
Δ MSNAf (bursts/min)	+5.5 ± 4.6	+4.2 ± 5.2	14	+2.8 ± 5.0	+0.1 ± 4.9	11
Δ MSNAi (bursts/100 hb)	+7.9 ± 7.0	+6.1 ± 7.8	14	-3.7 ± 9.4	+1.6 ± 7.3	11
Δ Ang II (ng/L)	+1.9 ± 2.4	+5.3 ± 20.5	26	+0.9 ± 1.6	-3.9 ± 15.2	26
Δ NE (ng/L)	+56 ± 54	+57 ± 34	26	+24 ± 59	+48 ± 52	24
Δ E (ng/L)	+2.8 ± 13.2	+5.1 ± 12.9	26	+25 ± 35	+21 ± 22	25

Abbreviations as in Table 1.

Values are means ± SD.

* $P < .05$; † $P < .01$.

nificantly increased sympathetic activity to skeletal muscle vasculature and the heart as well as norepinephrine levels in young male subjects. As anticipated, eprosartan led to a reduction of arterial pressure and markedly elevated Ang II plasma levels.

Peripheral Eprosartan Effect on Sympathetic Activity

It has been demonstrated in the pithed rat that eprosartan is able to reduce BP elevation caused by sympathetic stimulation at the spinal level.⁷ These investigators concluded that this compound—besides its AT1 antagonism at the vascular wall—blocks presynaptic AT1 receptors of sympathetic nerve endings to inhibit norepinephrine release.

In contrast, our results may question the proposed mechanism of lowering norepinephrine release by blocking presynaptic AT1 receptors in humans. There are investigations supporting our findings,^{20–22} as well as contradicting results.²³ The majority of the results showing that AT1 receptor antagonism is able to suppress sympathetic transmission has been obtained under conditions of sympathetic activation. To imitate those conditions we performed LBNP and mental stress, but failed to confirm blunted release of catecholamines in humans.

Central Eprosartan Effect on Sympathetic Activity

Besides the lack of peripheral dampening of sympathetic transmission, our data even suggest central sympathoactivation. At first sight, the two ways of action are conceivable. First, the BP reduction causes sympathoexcitation by baroreflexes, and second, components of baroreflex pathways are directly or indirectly affected by the high plasma levels of Ang II, as they occurred with AT1 receptor blockade in our study, as well as in others.²⁴ In our opinion, it is unlikely that elevation of central sympathetic

traffic by eprosartan is the effect of arterial baroreceptor unloading. This assumption is based on the following facts. First, as our data show, the lowering of diastolic arterial BP—representing the main determinant of baroreflexly triggered sympathetic bursts²⁵—versus the placebo treatment is very small (3 mm Hg) as compared to the elevation of MSNA by 36%. Notably, during section Rest 2 of our protocol there was no significant difference between the eprosartan and placebo session in arterial pressure, but a significantly higher MSNA (by 55%). Second, baroreflexes should exhibit an almost complete resetting after 1 week of AT1 receptor blockade, hence, regulating BP around the lower level (“leftward” resetting). In fact, Noll and co-workers¹ found a decrease in MSNA despite lowering of diastolic pressure by captopril even in an acute approach. Comparable results were achieved in rats, where the baroreflex relationship between arterial pressure and lumbar sympathetic nerve activity was shifted to lower pressures during ACE inhibition by enalapril, independent of whether or not baseline arterial pressure was restored by infusion of phenylephrine.²⁶ These findings suggest that ACE inhibitors are capable of shifting baroreflex set points to lower pressure ranges preventing an increase of sympathetic outflow despite lowered arterial pressure. Can this be attributed to inhibition of Ang II formation?

Numerous reports in the literature indicate that Ang II causes a resetting of baroreflexes, including altered autonomic drive to the heart, skeletal muscle, and kidney. In these studies, the effects of centrally applied Ang II depend on the particular site: shifts of baroreflex set points occur to the right^{27–29} as well as to the left.^{28,30}

Angiotensin II, however, is not able to pass the blood-brain barrier unless present in high concentrations.³¹ Therefore, data on effects of peripheral Ang II administration are of special interest concerning our study. In rabbits, intravenous infusion of Ang II caused significantly less reflex bradycardia and less inhibition of lumbar sym-

pathetic nerve activity than equipressor phenylephrine infusion,³² suggesting a rightward shift of the baroreflex curve by Ang II. In addition, these researchers excluded Ang II effects on the afferent and efferent limb of the baroreflexes and inferred a central action. That exogenous Ang II also evokes neurally mediated vasoconstriction by a central action has been demonstrated in rats.³³ In humans, Matsukawa et al³⁴ tried to offset the Ang II-evoked increase in arterial and central venous pressure by simultaneous infusions of nitroprusside. They succeeded in keeping central venous pressure at baseline levels but arterial pressure still tended to be higher. Nevertheless, MSNA was significantly higher with Ang II and nitroprusside as compared to phenylephrine and nitroprusside, strongly suggesting that Ang II stimulates sympathetic outflow.

What could be the mechanisms by which circulating Ang II resets baroreflex modulation of sympathetic activity at a central level? A recent review by DiBona³⁵ favors action of Ang II through circumventricular organs, specialized central nervous system areas in which the normal blood–brain barrier is lacking and being rich in AT1 receptor binding sites. Projections, finally reaching medullary and spinal sympathetic centers, cause elevation of sympathetic outflow. Alternatively, the increase of other angiotensins, directed to binding sites that are not blocked by eprosartan might explain our results.

Limitations of this Study

In our investigation, eprosartan was applied at the usual dose for only 1 week. It is conceivable that long-term administration might dampen sympathetic activity, bearing in mind that its full antihypertensive potential is achieved only within 2 to 3 weeks. On the other hand, it seems unlikely that a higher dosage alone would decrease sympathetic activity. Second, only young men with arterial BP <160/100 mm Hg were tested. Hence, our results may not apply directly to patients with moderate-to-severe forms of hypertension or suffering from heart or renal failure. Furthermore, plasma levels of norepinephrine do not exactly and not solely mirror sympathetic vasoconstrictor activity to skeletal muscle. Thus, inhibition of norepinephrine release to a certain degree cannot be excluded entirely.

Conclusion

These results provide direct evidence that administration of the AT1 receptor blocker eprosartan leading to BP reduction causes augmented central neural vasoconstrictor outflow and cast doubt on its ability to dampen norepinephrine release from peripheral sympathetic nerve endings in humans. We hypothesize that the marked elevation of Ang II plasma levels with AT1 receptor blockade may account for this decrease in central sympathetic restraint. It remains to be determined whether this sympathetic acti-

vation is of any significance in long-term treatment in hypertensive patients.

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