Central angiotensin II receptors mediate hemodynamic response variability to stressors

Kayla D. Rowe, Julie A. Schwartz, Lance L. Lomax, and Mark M. Knuepfer

Department of Pharmacological and Physiological Science, St Louis University School of Medicine, St. Louis, Missouri

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Central angiotensin II receptors mediate hemodynamic response variability to stressors. Am J Physiol Regul Integr Comp Physiol 291: R719–R727, 2006. First published April 6, 2006; doi:10.1152/ajpregu.00825.2005.—We examined whether ANG II receptors in the central nervous system mediate hemodynamic responses to pharmacological (cocaine) and behavioral (cold water) stressors. After administration of cocaine (5 mg/kg iv), rats were classified as vascular responders (VR) if their pressor response was due entirely to an increase in systemic vascular resistance (SVR) despite a decrease in cardiac output (CO). Cocaine elicited a pressor response in mixed responders (MR) that was dependent on small increases in both SVR and CO. ANG II (30 ng/5 μl icv, 5 min before cocaine) augmented the decrease in CO in VR and prevented the increase in CO in MR. Administration of [Sar¹,Thr⁸]ANG II (20 μg/5 μl icv; sarthran) before cocaine attenuated the decrease in CO and the large increase in SVR in VR so that they were no longer different from MR. Losartan (20 μg icv) or captopril (50 μg icv) preceding cocaine administration also attenuated the decrease in CO and the large increase in SVR seen in VR only. The role of angiotensin was not specific for cocaine, because ANG II (icv) pretreatment before startle with cold water (1 cm deep) enhanced the decrease in CO and the increase in SVR in both MR and VR, whereas losartan (icv) pretreatment before startle attenuated the decrease in CO and the increase in SVR in VR so that they were no longer different from MR.

These data suggest that central ANG II receptors mediate the greater vascular and cardiac responsivity in vascular responders to acute pharmacological and behavioral stressors.

COMPLEX PHYSIOLOGICAL RESPONSES can be evoked by pharmacological and behavioral stressors. Cocaine is a pharmacological stressor that elicits characteristic autonomic responses including increases in arterial pressure and redistribution of blood flow (16, 31). Cocaine use also has been correlated with a greater incidence of cardiovascular disease (16). Some individuals appear quite sensitive to cardiac toxicity even with low doses (16, 31). Behavioral stressors elicit hemodynamic response patterns that are strikingly similar to those evoked by cocaine (20, 21). Stressors, like cocaine, have been suggested to trigger sudden cardiac death, stroke, and hypertension in susceptible individuals (8, 11, 27). Many investigators have suggested that predisposition to stress-induced cardiovascular disease is due to excessive sympathoexcitatory responsiveness in some individuals (4, 7, 12). Therefore, there is considerable variability in individual responsiveness to disparate stressors.

Investigators in our laboratory have demonstrated that animals also have predetermined differences in hemodynamic response patterns to cocaine or behavioral stressors (18, 21). Our group reported wide variability in the cardiac output (CO) and systemic vascular resistance (SVR) responses to cocaine in conscious rats that is related to the incidence of hypertension and cardiovascular disease (20). Although all rats have similar pressor responses if cocaine induced a pressor response that was mediated by a large increase in SVR despite a decrease in CO. In contrast, rats were named mixed responders if the pressor response to cocaine was due to small increases in SVR and CO.

Vascular responders have greater cocaine-induced sympathoexcitation (2, 38) and are more prone to hypertension, cardiomyopathies, and toxicity (1, 17, 18, 44). The varying response patterns are not selective for cocaine, because other psychoactive agents (e.g., amphetamine, ethanol) or conditioned or unconditioned stressors elicit similar response variability (1, 20, 21). Some humans respond to acute behavioral stressors with a decrease in CO, and these individuals are more likely to develop hypertension and heart disease (10, 43). Therefore, our group proposed that the rat may be an appropriate model for studying predisposition to cardiovascular disease (20).

Angiotensin II (ANG II) is a vasoactive peptide involved in fluid volume regulation and electrolyte homeostasis (37). ANG II produces an increase in arterial pressure due to both a direct vasoconstrictor effect, mediated by AT₁ receptors in blood vessels, and a central sympathoexcitatory effect. Peripheral ANG II binds to receptors in the circumventricular organs of the brain, including the organum vasculosum of the lamina terminalis and the subfornical organ (29). These sites, in turn, send projections to several other nuclei that activate the sympathetic nervous system, increasing arterial pressure and regulating fluid balance. There also is evidence of a central renin-angiotensin system (RAS) that contributes to stress responses (6, 23, 41). Endogenous ANG II can be found in numerous periventricular locations (29), and ANG II receptors (both AT₁ and AT₂) are located within the hypothalamus, brain stem, and pituitary (45). The central RAS also has been implicated in long-term regulation of arterial pressure and the etiology of hypertension (3, 35, 36, 42).

We propose that the varying hemodynamic response patterns of vascular and mixed responders are produced by differences in central ANG II receptor activation in these rats. Therefore, we hypothesize that centrally administered ANG II exacerbates hemodynamic responses to cocaine and to startle, and that...
centrally administered ANG II antagonists or angiotensin-converting enzyme (ACE) inhibitors can prevent the differences in hemodynamic responses seen between vascular and mixed responders. This, in turn, would suggest that central ANG II receptors may be responsible for the predisposition of vascular responders to develop hypertension and heart disease.

METHODS

Surgical Preparation

These experiments were performed in conscious, instrumented rats. All surgical and experimental procedures were approved by the St. Louis University Institutional Animal Care and Use Committee and followed guidelines described in the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington, DC, 1996).

Specific pathogen-free, male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 300–350 g were instrumented for measurement of cardiac output (CO) with the use of miniaturized pulsed-Doppler flow probes (Iowa Doppler Products, Iowa City, IA) placed on the ascending aorta and with intracerebroventricular guide cannulas for central nervous system (CNS) administration of drugs as described earlier (1, 17–19, 21). After a recovery period (7–10 days), the femoral artery and vein were cannulated for arterial pressure determination and intravenous drug infusion, respectively. All surgical instrumentation was performed under deep anesthesia (isoflurane) using aseptic technique. Antibiotic treatment (cefazolin, 1 mg/kg) and analgesic treatment (buprenorphine, 0.05 mg/kg) were administered after surgery. Rats that lost >10% body weight and those that did not have normal locomotor and behavioral responses 2–3 days after surgery were euthanized with pentobarbital (70 mg/kg iv).

Experimental Preparation

Pharmacological stress with cocaine. Two to three days later, arterial pressure, heart rate, and CO were recorded before and after administration of cocaine (5 mg/kg iv). Cocaine was administered five to seven times (no more than twice daily) while hemodynamic responses were recorded for 5 min. The average responses over the first 60 s (peak pressor response and 15, 30, 45, and 60 s after the initial increase in arterial pressure) were used to characterize the rats as vascular or mixed responders. Those rats with an increase in CO were designated mixed responders, whereas those with a decrease were vascular responders. This procedure has been used previously in our laboratory and described in detail previously (1).

After characterization, cocaine (5 mg/kg iv) was administered 5 min after an infusion of saline (10 μl iv) injected over 2 min. A minimum of 3 h later, rats were treated with ANG II (10 or 30 μg) or an ANG II antagonist (sarathran, losartan, or captopril) (10 μl total, 2-min infusion including a saline flush) intracerebroventricularly 5 min before cocaine (5 mg/kg iv) administration while hemodynamics were recorded.

Behavioral stress with cold water. Most of the rats used for testing with cocaine were also tested with exposure to cold water. Rats were placed in a Plexiglas cage (24 × 24 × 45 cm high) designed with a narrow chute to channel water added from the top to the bottom of the cage by gravity. After acclimation for at least 3 h, cold water was quickly added to the cage bottom (3–5°C, 1 cm deep) through the chute, minimizing the spread of water to areas other than the paws and limbs of the rat. After 1 min, water was rapidly drained from the cage through a hole at its base. Rats were allowed to recover at least 30 min or longer if hemodynamic values had not returned to normal. The hemodynamic responses to cold stressor trials were tested four to ten times with no more than five trials per day in each rat.

After at least three trials to obtain control startle responses, rats were given an injection of vehicle (0.9% saline, 10 μl iv) 5 min before cold water exposure. After a minimum of 30 min for recovery, rats were given losartan (20 μg icv) or ANG II (30 μg icv) 5 min before cold water exposure for 60 s.

Drugs and Chemicals

The specific agents used were ANG II (10 and 30 ng), [Sar1,Thr8]ANG II (sarathran, 20 μg), a nonspecific ANG II antagonist, and captopril (50 μg), an ACE inhibitor, which were obtained from Sigma (St. Louis, MO). Losartan (20 μg), a selective AT1 receptor antagonist, was kindly provided by Merck (Whitehouse Station, NJ). Cocaine hydrochloride was obtained from the National Institute on Drug Abuse and dissolved in normal saline. Other drugs used during the experiment include isoflurane (Baxter Pharmaceutical Products, Deerfield, IL), cefazolin (Geneva Pharmaceuticals/Marsam Pharmaceuticals, Cherry Hill, NJ), buprenorphine (Henry Schein, Melville, NY), and heparin (1,000 U/ml; Elkins-Sinn, Cherry Hill, NJ).

Data Analysis

Data were analyzed from digital files using WINDAQ software (DAQTIQ Instruments, Akron, OH). A three-way, repeated-measures analysis of variance (ANOVA) was performed to examine the effects of intracerebroventricular drug administration, the effect of each drug over time (first 60 s), and the differences between vascular and mixed responders in response to cocaine. Responses to cold water stress were divided into two groups. The initial startle response (during first 5 s) was analyzed with a two-way ANOVA (effects in vascular and mixed responders and effects of intracerebroventricular drug administration). A post hoc Bonferroni’s test was used to examine individual differences. The delayed cold pressor response (15–60 s) was analyzed with a three-way ANOVA similar to the data with cocaine exposure except that the initial peak was not included. Statistics were performed using GBStat (Dynamic Microsystems, Silver Springs, MD). Significant changes were assumed if P < 0.05.

RESULTS

Pharmacological Stress with Cocaine

Control values and responses. Rats (n = 30) examined in this experiment had a mean arterial pressure of 115 ± 3 mmHg, a heart rate of 387 ± 7 beats/min, and an ascending aortic flow of 9.6 ± 0.4 kHz shift. There were no significant differences in the resting values of vascular and mixed responders for arterial pressure (114 ± 5 and 116 ± 3 mmHg, respectively), heart rate (386 ± 7 and 387 ± 15 beats/min, respectively), or ascending aortic blood flow (9.2 ± 0.5 and 10 ± 0.8 kHz, respectively).

The average responses over the first 60 s following cocaine administration (peak pressor response and 15, 30, 45, and 60 s after the initial increase in arterial pressure) were used to characterize the rats as vascular or mixed responders. Those rats with an increase in cardiac output were designated mixed responders, whereas those with a decrease were vascular responders. This procedure has been used previously in our laboratory and described in detail previously (1).

After characterization, cocaine (5 mg/kg iv) was administered 5 min after an infusion of saline (10 μl iv) injected over 2 min. A minimum of 3 h later, rats were treated with ANG II (10 or 30 μg) or an ANG II antagonist (sarathran, losartan, or captopril) (10 μl total, 2-min infusion including a saline flush) intracerebroventricularly 5 min before cocaine (5 mg/kg iv) administration while hemodynamics were recorded.

Behavioral stress with cold water. Most of the rats used for testing with cocaine were also tested with exposure to cold water. Rats were placed in a Plexiglas cage (24 × 24 × 45 cm high) designed with a narrow chute to channel water added from the top to the bottom of the cage by gravity. After acclimation for at least 3 h, cold water was quickly added to the cage bottom (3–5°C, 1 cm deep) through the chute, minimizing the spread of water to areas other than the paws and limbs of the rat. After 1 min, water was rapidly drained from the cage through a hole at its base. Rats were allowed to recover at least 30 min or longer if hemodynamic values had not returned to normal. The hemodynamic responses to cold stressor trials were tested four to ten times with no more than five trials per day in each rat.

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experiments. Administration of ANG II (10 μg icv), before cocaine administration, increased arterial pressure without affecting other variables (Table 1). Pretreatment with ANG II (10 μg icv) followed by cocaine administration (5 mg/kg iv) did not result in any statistically significant changes in either group.

Administration of ANG II (30 μg icv) before cocaine administration elicited an increase in arterial pressure and SVR in both groups of rats (Table 1). Pretreatment with ANG II (30 μg icv) attenuated the increased CO in mixed responders, while exacerbating the decreased CO in vascular responders (Fig. 2).

Pretreatment with antagonists. Administration of captopril (50 μg icv) before cocaine administration did not alter hemodynamic responses to cocaine (5 mg/kg iv) in either group after cocaine administration but pretreatment with sarthran (20 μg icv) produced a decrease in heart rate in both groups (Table 1). Pretreatment with sarthran attenuated the pressor response to cocaine administration (5 mg/kg iv) in both groups of rats (Fig. 4). However, the effect of sarthran on arterial pressure was significant only for the vascular responders and was mediated by a decrease in SVR. Sarthran pretreatment additionally eliminated the differences in CO and heart rate responses following cocaine administration between vascular and mixed responders (Fig. 4).

Administration of losartan (20 μg icv), an AT1 receptor antagonist, before cocaine administration did not alter any hemodynamic values measured (Table 1). Losartan pretreatment did not alter hemodynamic responses to cocaine (5 mg/kg iv) in mixed responders, the ACE inhibitor attenuated both the decrease in CO and the increase in SVR in vascular responders such that their hemodynamic response profile to cocaine was no longer different from that of mixed responders.

Table 1. Effects of pretreatment on resting values (5 min after administration)

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<th>Dose</th>
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<th>Group</th>
<th>MAP Change, mmHg</th>
<th>HR Change, beats/min</th>
<th>CO Change, %</th>
<th>SVR Change, %</th>
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<tr>
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<td>6 ± 4</td>
<td>2 ± 5</td>
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<td>MR</td>
<td>3 ± 2</td>
<td>2 ± 13</td>
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Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; SVR, systemic vascular resistance; SV, stroke volume; MR, mixed responders; VR, vascular responders. *P < 0.05, significant change from control (saline) responses as determined by 1-way ANOVA.
vented the differences in CO and SVR between mixed and vascular responders (Fig. 5).

Behavioral Startle with Cold Water

**Control values and responses.** In this experiment, rats \( n = 24 \) had a mean arterial pressure of 119 ± 2 mmHg, a heart rate of 403 ± 5 beats/min, and an ascending aortic flow of 11.2 ± 0.4 kHz shift. There were no significant differences in the resting values of vascular and mixed responders to startle before presentation of the cold water. Cold water startle alone (within first 5 s of adding cold water) produced similar pressor and flinch responses in vascular and mixed responders, but differences in hemodynamic response profiles existed (Fig. 6). The pressor response in mixed responders was due to small increases in SVR and CO, whereas the pressor response in vascular responders was dependent only on a large increase in SVR while CO decreased. Only the initial startle responses to cold water resembled the varying response profiles of vascular and mixed responders to cocaine administration (Fig. 1).

**Pretreatment with agonist.** ANG II pretreatment (30 μg icv) before cold water startle elicited increases in arterial pressure in mixed and vascular responders (Table 1) without affecting other variables. ANG II pretreatment altered responses to cold water startle in mixed responders but not in vascular responders. Startle produced a decrease in CO and stroke volume along with a greater increase in SVR in mixed responders so that their hemodynamic response profile became nearly identical to responses in vascular responders (Fig. 7). ANG II pretreatment depressed the delayed CO and heart rate responses to cold water exposure.

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**Fig. 2.** Responses to cocaine (Coc; 5 mg/kg iv) 5 min after ANG II administration (Ang; 30 ng icv) in vascular responders (VR; \( n = 5 \)) and mixed responders (MR; \( n = 7 \)). #Significant differences between VR and MR. *Significant differences due to drug pretreatment.

**Fig. 3.** Responses to cocaine (5 mg/kg iv) 5 min after captopril (Capt; 50 μg icv) administration in VR and MR \( (n = 6 \text{ and } 7, \text{ respectively}) \).
Pretreatment with antagonist. Losartan (20 μg icv) pretreatment before cold water startle did not alter hemodynamic variables (Table 1). Losartan pretreatment did not significantly alter the hemodynamic responses to startle in vascular or mixed responders but prevented the difference in CO and SVR responses between these groups (Fig. 8). Specifically, in vascular responders, losartan administration attenuated the increase in arterial pressure by attenuating the increase in SVR in response to startle. The delayed response (15–60 s) was not affected, and differences between vascular and mixed responders in the CO and stroke volume responses were maintained (Fig. 8).

DISCUSSION

Consistent with previous studies (9, 45), our data suggest that central ANG II receptors mediate the hemodynamic responses to stressors. Moreover, the results suggest that ANG II receptors are responsible for hemodynamic response variability between mixed and vascular responders. This is a novel finding that suggests differences in central neurochemistry between these groups. The results also corroborated earlier observations suggesting that hemodynamic response profiles to a pharmacological stressor (cocaine) and to a behavioral stressor (startle) were similar and demonstrated that vascular responders to either stressor had a similar dependence on central AT1 receptors. Finally, these data support the hypothesis that one or more of the central sites containing AT1 receptors mediates the greater vascular responsiveness to stressors in vascular responders and, possibly, the predisposition to develop cardiovascular disease that exists in vascular responders. Each of these findings is discussed.

Others have reported that central angiotensin receptors play a role in mediating hemodynamic responses to stressors. Cen-
central ANG II receptor blockade attenuated the pressor response induced by foot shock and heat stressors in rats (6, 23). Losartan (icv) attenuated both the tachycardia and pressor responses to immobilization in a dose-dependent manner (41). Under acute immobilization, Saiki et al. (41) suggested that endogenous ANG II binds to AT1 receptors in the brain, leading to activation of sympathetic nervous and adrenomedullary systems and inducing tachycardia. Therefore, they proposed that ANG II could be critical for stressor-induced hemodynamic responses in a manner similar to corticotropin-releasing factor (CRF) and arginine vasopressin (AVP; Ref. 41). They also proposed that adaptation to chronic immobilization could be due to decreased secretion of AVP and/or CRF or decreased sympathetic nervous activity (41). ANG II also plays a role in stressor-induced stimulation of adrenocorticotropin hormone release by eliciting CRF release (9). Further, in situ hybridization recently showed that both acute and chronic exposure to air jet or immobilization stressors increased AT1 mRNA in the paraventricular nucleus, an area involved in the release of AVP and CRF (9). Therefore, the observation that central ANG II plays a role in stress responsiveness is primarily corroborative.

Our results suggest AT1 receptors are critical for the differences in response profiles between vascular and mixed responders due to several observations. First, ANG II administration (icv) exacerbated the decrease in CO following cocaine administration in vascular responders and prevented an increase in CO to cocaine in mixed responders. Therefore, after ANG II administration, mixed responders no longer had smaller increases in systemic vascular resistance and increases in CO, making them similar to vascular responders. Second, intracerebroventricular pretreatment with ANG II receptor an-

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**Fig. 6.** Initial startle response to behavioral stress with cold water (1 cm deep) in VR and MR (n = 18 and 6, respectively). Significant differences (P < 0.01) between VR and MR.

**Fig. 7.** Responses to cold water after saline pretreatment (CS) compared with responses 5 min after ANG II (30 ng icv) in VR and MR (n = 10 and 5, respectively). #Significant differences between vascular and mixed responders. *Significant differences due to drug pretreatment.
agonists, losartan and sarthran, or captopril, an ACE inhibitor, before cocaine administration prevented the differences in hemodynamic response profiles in mixed and vascular responders specifically by preventing the greater vasoconstriction and/or cardiac depression noted in vascular responders. Finally, losartan also prevented the vascular responder phenotypic response pattern to cocaine or startle, implicating central AT1 receptors specifically for the greater vascular responsiveness to stressors. This evidence suggests that the differences between the vascular and mixed responder response profiles are mediated, at least in part, by central ANG II reactivity. We do not know of any evidence in the literature suggesting that acute response variability to stressors is dependent on differences in AT1 receptors, but this seems to be a distinct possibility considering these results.

Our group has reported that hemodynamic response patterns to cocaine and to startle with cold water are similar in magnitude and pattern (21). The results of the startle experiments following ANG II and losartan administration show similar alterations in the hemodynamic response profiles to the studies examining cocaine-induced hemodynamic responses after pretreatment with each drug. For example, ANG II produced a significant decrease in CO and an increase in SVR in mixed responders after startle elicited by cold water so that their initial response to startle was no longer different from that of vascular responders. We proposed that the central neural pathways mediating acute responses to cocaine or to behavioral stressors may be similar to those differences that make vascular responders more prone to cocaine-induced cardiomyopathies and to repeated cocaine-or stress-induced sustained increases in hypertension (1, 17, 20, 32). Gaudet et al. (13) showed that chronic oral administration of losartan reduced the pressor response to stressors in spontaneously hypertensive rats, suggesting that peripheral and/or central AT1 receptors mediate increases in arterial pressure after exposure to stressors. It remains to be determined whether central AT1 receptors are responsible for greater susceptibility to cardiovascular disease in vascular responders.

The present studies cannot specifically indicate what site in the brain mediates the effects on vascular and cardiac responsiveness, but several regions, particularly periventricular, are possible. ANG II receptors have been identified at many sites in the rat brain, both within and outside the blood-brain barrier (5). Very high densities of ANG II receptors are located in the subfornical organ, paraventricular, and periventricular nuclei of the hypothalamus, the nucleus of the solitary tract (NTS), and the area postrema (30). The brain AT1 receptor subtype is found in several circumventricular organs, the paraventricular nucleus of the hypothalamus, and the NTS, rostral ventrolateral medulla (RVLM), and dorsal motor nucleus of the vagus nerve, all areas that regulate cardiovascular responsiveness (14, 29, 34, 41). The AT1 receptor, which has been largely characterized on the basis of its high affinity for losartan, is suggested to be responsible for most of the known physiological actions of ANG II (24). A second type of ANG II receptor, AT2, is localized in a number of brain areas that are not associated with cardiovascular responses, including the thalamus, inferior olivary nucleus, and cerebellar cortex (14, 25, 34, 40). Our studies have not differentiated between brain stem and diencephalic sites of action, but ANG II affects arterial pressure at both sites. Endogenous ANG II acts at AT1 receptors of the NTS to attenuate the baroreceptor reflex in rats (28). Microinjection of ANG II into the RVLM elicits an increase in arterial pressure and sympathetic activity (15). In contrast, unilateral microinjections of sarthran into the RVLM result in a moderate fall in arterial pressure and sympathetic activity in the rat (33). Hypothalamic regions such as the median preoptic nucleus (mnPO) also may be involved, because preliminary observations suggest that microinjection of losartan in the mnPO prevented the characteristic hemodynamic responses in vascular responders with little change in mixed responders, suggesting that AT1 receptors in the mnPO may mediate the differences in responsivity between mixed and vascular responders (39).

Administration of ANG II produced a sustained increase in arterial pressure. The pressor response was dependent on an
increase in systemic vascular resistance in one group of rats (cocaine treated), whereas there was not a significant increase in vascular resistance in a different group of rats (cold water treated; Table 1). Although this appears anomalous, the average of arterial pressure and SVR over the 5 min was significantly elevated (data not shown). Therefore, the pressor response to intracerebroventricular ANG II actually occurred before the 5-min time period and was related to a significant increase in SVR, because CO did not change. We also noted in Table 1 that ANG II receptor blockade or converting enzyme inhibition did not alter the arterial pressure, suggesting that the central maintenance of sympathetic tone to the vasculature is not tonically mediated by ANG II.

In conclusion, we have provided additional evidence for the participation of central ANG II in the hemodynamic responses to pharmacological and behavioral stressors. In addition, our results suggest that AT1 receptors in the CNS may be responsible for differences in acute hemodynamic responsiveness. More interestingly, we propose that central AT1 receptors may be responsible for the differences in predisposition to cardiovascular disease. It is believed that ANG II may contribute to the development of experimental hypertension through actions in the mnpO (3). We reported that vascular responders are more likely to develop cocaine- or stress-induced hypertension (1, 20, 32). Therefore, the acute response patterns (vascular or mixed) and possibly the long-term predisposition to develop hypertension are dependent on activation of central angiotensin receptors. The specific location of these receptors is not yet known, but the median preoptic nucleus, the subfornical organ, the organum vasculosum of the lamina terminalis, and the paraventricular nucleus are likely possibilities, because they contain angiotensin receptors and play a role in central fluid volume and arterial pressure regulation (29, 36). Future studies to determine locations within the angiotensinergic signaling pathway that differ between vascular and mixed responders should further characterize the reasons for their differential response patterns to stressors.

ACKNOWLEDGMENTS

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DISCLOSURE

Preliminary reports of these studies were published in abstract form (26) and as part of a review article (22).

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REFERENCES


