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BRIEF COMMUNICATION

Hypotensive Effects of Sarthran in Normotensive and Spontaneously Hypertensive Rat Strains

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MURRAY, C. E., J. W. HARDING AND J. W. WRIGHT. *Hypotensive effects of sarthran in normotensive and spontaneously hypertensive rat strains*. PHARMACOL BIOCHEM BEHAV **39**(4) 1029–1032, 1991.—The specific angiotensin receptor antagonist [Sar[1],Thr[8]]AII (sarthran) was intracerebroventricularly (ICV) infused in anesthetized spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) normotensive controls. The results extend earlier findings by determining that: 1) the hypotensive effect of ICV-infused sarthran could be enhanced in anesthetized as compared with alert animals; 2) SHRs revealed a greater hypotensive response as compared with WKY rats; and 3) no sarthran-induced agonistic effects were observed in contrast with previous results using alert SHRs. These findings support the use of sarthran as a potent angiotensin receptor antagonist to investigate the role of the brain angiotensin system in the control of normal and dysfunctional blood pressure.

Arterial pressure Sarthran Intracerebroventricular infusions Hypotension Spontaneously hypertensive rat

A dysfunctional brain angiotensin system appears to be a major contributor to the high blood pressure observed in the spontaneously hypertensive rat (SHR) model of human essential hypertension (3,10). In this model, intracerebroventricular [ICV; (4, 8, 24)] and intraarterial (23) injections of angiotensin II (AII) and III (AIII) produce exaggerated pressor responses as compared with Wistar-Kyoto (WKY) normotensive rats. There are additional findings in the SHR of deficiencies in brain aminopeptidase activity (25), and elevations in angiotension levels (14,16) and AII binding sites (5) in relevant cardiovascular structures in the brains of SHR. Consistent with these observations, electrophysiological studies indicate a significantly increased sensitivity to microiontophoretically applied AII and AIII in the paraventricular nucleus of the hypothalamus of SHR as compared with WKY rats (6).

The present study was designed to evaluate the efficacy of an ICV-infused specific angiotensin receptor antagonist, sarthran, to reduce blood pressure in the SHR. We reasoned that, if the heightened sensitivity of the SHR to angiotensins was dependent upon an elevation in number of brain angiotensin receptors and/or increased levels of angiotensin, then the application of an angiotensin receptor antagonist would be expected to reduce blood pressure to a greater degree in the SHR as compared with the WKY rat. We selected sarthran over other available angiotensin receptor antagonists because it has been reported to possess less agonistic activity in normotensive rats when delivered ICV (15), although an initial agonistic effect has been observed to ICV-infused sarthran in the SHR (9).

METHOD

Adult male SHR and WKY rats (120–150 days of age) were derived from breeding stock obtained from Taconic Farms (Germantown, NY) and were maintained in group cages in a AAALAC-accredited vivarium on a 12:12-h photoperiod initiated at 0700 h at a temperature of 21–22°C. Each animal was tranquilized with diazepam (2.5 mg/kg IM, Sigma Chemical, St. Louis, MO) followed 10 min later by ketamine anesthesia (100 mg/kg IM with 5-mg boosts at 30-min intervals (Bristol Laboratories, Syracuse, NY), prepared with a left femoral artery catheter (PE-50, Clay Adams) and an ICV guide cannula (PE-60) stereotaxically positioned with the beveled tip above the roof of the right lateral ventricle, as previously described (24). Sarthran [Sar[1], Thr[8]]AII was purchased from Sigma Chemical Company (A9900 Lot #93F-5805) and was 80% pure by weight as measured by HPLC. Acetate salts represented the major contributor to the decreased purity by weight. Peptide purity was estimated to be 98% by Sigma Chemical Company.

Sarthran infusion was initiated after a stable blood pressure was established for a minimum of 10 min following anesthetization. This was accomplished by inserting a prefilled 24-ga stainless steel hypodermic tubing injector into the ICV guide cannula such that the injector extended 2–3 mm beyond the tip of the guide, thus penetrating the roof of the lateral ventricle. The injector was connected to a 10-μl Hamilton syringe by a 30-cm length of PE-20 tubing, and the syringe was driven at a flow rate of 2 μl/min (Sage Instruments infusor, Model 355). Sar-

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thran at doses of 0, 1, 10 and 20 nmol/2 μl/min for 10 min was delivered to each animal, with sufficient time between doses to recover stable baseline blood pressure. A minimum of 30 min to recover baseline blood pressure was allowed following the 1 and 10 nmol/min doses, while 90 min were allowed following the 20 nmol/min dose. Maximum blood pressure decreases usually occurred during the 15 min following the termination of infusion. One-half of the animals of each strain were administered the doses in an ascending order, while the remaining animals received the doses in a descending order. Changes in blood pressure were measured via the femoral artery catheter connected to a Statham transducer (Model PE23AC, Gould and Company, Inc., Oxnard, CA) and a Grass polygraph (Model 5D). The magnitude of the pressor responses was calculated by subtracting the corresponding pretreatment mean arterial blood pressure (MABP) from the maximum change during the 10-min infusion and 30-min postinfusion periods. At the highest dose (20 nmol/min), maximum changes during each 5-min period during the 80 min following infusion were also measured. The infusion data were analyzed by a 2 (Strain) × 4 (Dose) analysis of variance (ANOVA) with repeated measures on the second factor. In order to evaluate for the order of treatments, the results from ascending and descending doses for the SHR and WKY groups were each analyzed by a two-factor (Subgroups and Doses) mixed-design ANOVA with repeated measures on doses. Significant effects were further evaluated by Newman-Keuls post hoc tests, with the level of significance set at 0.01. The percent change from baseline blood pressure data sets comparing SHR and WKY rats was analyzed by *t*-tests.

RESULTS

Intracerebroventricular infusion of sarthran reduced blood pressure in members of both strains in a dose-response fashion; these decreases were greater in the SHR as compared with the WKY normotensive controls (Fig. 1). Specifically, there were significant differences across strains, $F(1,14)=8.28$, $p<0.02$, with the SHR revealing greater overall decreases than WKY rats. As expected, there was a dose effect, $F(3,42)=37.72$, $p<0.0001$, with increasing doses producing greater declines in absolute blood pressure that were each significantly different from the next, except for the 0 and 1 nmol/min doses that did not differ. The Strain × Dose interaction was also significant, $F(3,42)=3.07$, $p<0.05$. Post hoc analyses revealed that SHR responded with significantly greater decreases in blood pressure to the 10 and 20 nmol/min doses of sarthran as compared with the WKY rats. The SHR showed maximum mean (±SEM) decreases in blood pressure from baseline (177.8±2.1 mmHg) of 48.0 (±11.6) and 73.6 (±9.2) mmHg for the 10 and 20 nmol/min doses, respectively, while the WKY rats displayed declines from baseline (129.6±1.3 mmHg) of 26.3 (±7.6) and 44.3 (±4.7) mmHg, respectively. Since we were interested in comparing members of two rat strains with very different resting baseline blood pressures, it was deemed appropriate to also analyze percentage change from baseline. The SHR revealed maximum mean decreases of −27.0 and −41.4% for the 10 and 20 nmol/min doses, respectively. Comparable values for the WKY rats were −20.3 and −34.2%. These values for the 10 and 20 nmol/min doses were significantly different [$ts(14)=2.52$ and 2.74, respectively, $p<0.05$].

These strain differences are further illustrated in Fig. 2, which presents the pattern of blood pressure responses measured to the 0 and 20 nmol/min doses of sarthran. The SHR revealed a steady decline in MABP that continued during the postinfusion period until it reached a maximum drop of 73.6 mmHg at 20 min (10 min following determination of infusion). Total recovery time required to reestablish baseline pressure was 60 min. The WKY rats indicated a similar decline in blood pressure; however, the maximum decline was 44.3 mmHg at 25 min. The WKY animals required 30 min to recover baseline blood pressure following termination of infusion.

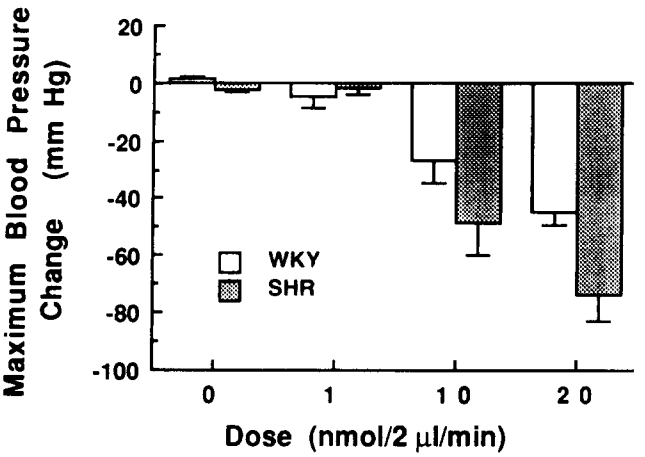

FIG. 1. Maximum changes in mean (±SEM) arterial blood pressure from baseline in response to ICV sarthran infusion at doses of 0, 1, 10 and 20 nmol/2 μl aCSF/min for 10 min. Each bar represents pooled data from 8 animals. Mean (±SEM) baseline blood pressures were 129.6 (±1.3) and 177.8 (±2.1) mmHg for the WKY and SHRs, respectively.

DISCUSSION

Previous investigations utilizing angiotensin receptor antagonists have had varying degrees of success in producing decreases in blood pressure. Saralasin has been reported to produce a mean decrease of 12.5 mmHg at an ICV dose of 20 μg (22 nmol) administered to stroke-prone SHR (13,17). [Sar^1,Ile^8]AII has been reported to decrease mean arterial blood pressure by approximately 20 mmHg following an acute dose of 100 μg (103 nmol) ICV in rats made hypertensive by aortic coarctation (20). Chronic infusion of [Sar^1,Ile^8]AII [1200 ng/h (1.2 nmol/h)] into the lateral ventricles of SHR produced an overall mean drop of 33 mmHg over the 6-day infusion period, with a maximum drop

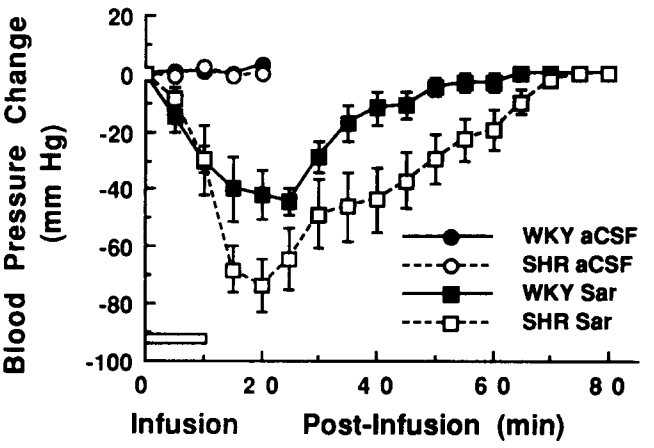

FIG. 2. Maximum changes in mean (±SEM) arterial blood pressure from baseline during each 5-min period during the 10-min ICV infusion of 0 or 20 nmol/2 μl aCSF/min sarthran, and 70 min following infusion. Mean (±SEM) baseline blood pressures were 133.6 (±2.8) and 175.6 (±4.9) mmHg for the WKY (N=8) and SHR (N=8) groups, respectively.

of approximately 55 mmHg at 18 hours into the infusion (12). Schoelkens et al. (19) administered an acute ICV injection of [Suc^1,Val^5,Phg^8] AII [300 ng/kg (0.3 nmol/kg)] and measured decreases of 8 mmHg in 10-week-old SHR; however, the same dose at 14 weeks of age produced an increase in blood pressure. The ICV injection of [Sar^1,Ile^8]AII, saralasin, or [Suc^1,Val^5,Phg^8] AII had no effect on blood pressure in normotensive rat strains.

Two previous reports concerned with ICV-infused sarthran in the SHR should be described. Bruner et al. (1) observed no significant decreases in blood pressure with chronic ICV infusion [1 μg/h for 14 days and 6 μg/h for 7 days (1.1 and 6.3 nmol/h, respectively)] of sarthran, although this antagonist did inhibit subsequent ICV AII-induced increases in blood pressure. Jensen et al. (9) reported that acute infusion of sarthran in alert SHR produced a maximum mean reduction of 35.2 mmHg, while Sprague-Dawley (SD) and WKY normotensive control rats revealed reductions in blood pressure of approximately 14 mmHg. An initial significant transient sarthran-induced agonist effect was measured in the SHR, but was not observed in normotensive SD and WKY rats. The differences in results obtained can perhaps be explained by the different methods used to measure blood pressure. Bruner and colleagues utilized the tail cuff method, while Jensen et al. prepared animals with an indwelling arterial catheter. Several investigations have indicated that SHR respond with exaggerated elevations in blood pressure to two requirements of the tail cuff technique, i.e., restraint and whole-body warming (2, 22, 26). Thus Bruner et al. (1) may have failed to observe sarthran-induced decreases in blood pressure due to the measurement method employed. A second possible explanation concerns sarthran-induced tachyphylaxis. It has been shown that repeated ICV injections of AII or AIII produce tachyphylaxis of the drinking response in rats (18). This decrease in responsiveness was shown to be due to a desensitization of angiotensin receptors with repeated stimulation (7). Given that Bruner et al. (1) administered sarthran chronically for 7 to 14 days, a sarthran-induced tachyphylaxis may have occurred negating the hypotensive effects observed when sarthran is administered acutely. Since no tachyphylaxis data are presently available from either peripheral or central application, this important issue remains to be resolved.

The use of diazepam followed by ketamine anesthesia in the present investigation allowed the achievement of baseline blood pressures approximately equivalent to those of calm, alert rats. Our results indicate that infusion of sarthran at the highest dose of 20 nmol/min produced maximum mean reductions in blood pressure of 73.6 mmHg in the HR, with individual animals revealing maximum reductions of up to 110 mmHg; WKY rats exhibited maximum mean decreases of 44.3 mmHg at this dose. The agonistic effect of ICV-infused sarthran previously reported in SHR (9) was not observed in either SHR or WKY rats under these experimental conditions. Previous research suggests that SHR are more reactive to novel or noxious stimuli and show less habituation to stressful situations (11,21). Thus, by utilizing anesthetized animals, reaction to environmental stimuli was greatly reduced, presumably resulting in less interference with sarthran-induced responses.

The present results, coupled with those from Jensen et al. (9) utilizing alert SHR and WKY rats, indicate that the specific angiotensin receptor antagonist, sarthran, may be a more effective hypotensive agent than saralasin, [Sar^1,Ile^8]AII, or [Suc^1, Val^5,Phg^8] AII when acute ICV infusions are employed. Sarthran produced dose-dependent decrements in blood pressure in animals pretreated with diazepam and anesthetized with ketamine. Under these experimental conditions, the transient agonistic effects previously reported in alert SHR (9) were not observed. It should be pointed out that both diazepam and ketamine are known to interact with central glutamatergic transmission and to influence N-methyl-D-aspartate receptors. Therefore, it is conceivable that the observed hypotensive effects are due to an interaction among diazepam, ketamine and sarthran. We believe this to be unlikely, given: 1) that the present declines in blood pressure were similar to those reported with ICV infusion of sarthran (10 nmol/min) in alert, free-moving SHR and WKY rats (9); 2) the temporal patterning of blood pressure decrease and recovery with the infusion of sarthran; and 3) that ketamine stimulates the sympathetic nervous system, which would oppose declines in blood pressure. However, since we have not attempted to replicate these findings using another anesthetic agent, this possibility cannot be ruled out.

In sum, these sarthran-induced responses support the notion that the brain angiotensin system is important to ongoing blood pressure regulation and that sarthran may be a valuable angiotensin receptor antagonist to establish the specificity of angiotensin-induced responses.

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