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Effects of Atrial Natriuretic Factor on Drinking Responses to Central Angiotensin II

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LAPPE, R. W., J. L. DINISH, F. BEX, K. MICHALAK AND R. L. WENDT. *Effects of atrial natriuretic factor on drinking responses to central angiotensin II.* PHARMACOL BIOCHEM BEHAV 24(6) 1573–1576, 1986.—The present study examined the effects of ANF(4–28)[Wy-47,663], a synthetic 25 amino acid human atrial natriuretic factor, on the dipsogenic actions of centrally-administered angiotensin II in conscious rats. Bolus injection (100 ng) or continuous infusion (60 ng/min) of Wy-47,663 or vehicle into the lateral cerebroventricle had no effect on mean arterial pressure or heart rate. No obvious behavioral changes were observed after central administration of Wy-47,663 or vehicle. Central injection of angiotensin II (15 or 30 ng) promptly elicited prolonged drinking responses in vehicle-treated rats. In rats pretreated with Wy-47,663, the onset of the angiotensin II-induced drinking responses was significantly delayed compared to vehicle-treated animals. However, Wy-47,663 had no effect on the total volume consumed over 30 minutes after angiotensin II injection. Intravenous infusion of Wy-47,663 (2μg/kg/min) failed to alter the dipsogenic action of centrally administered angiotensin II. These data indicate that atrial natriuretic factor found within the brain but not the peripheral circulation may participate in the regulation of extracellular fluid volume by modulating the dipsogenic actions of the central renin-angiotensin system.

Conscious rats Human ANF Intracerebroventricular administraiton Arterial pressure Heart rate

ATRIAL natriuretic factor (ANF) is a generic term referring to a group of peptides with analogous structures which were initially isolated from the atria of mammalian hearts [2,3]. The atrial peptides are potent natriuretic and diuretic agents with hypotensive properties (for reviews see [1, 9, 13, 16]). The depressor actions of ANF may be mediated, in part, through antagonism of the peripheral effects of angiotensin II. Previous investigators have reported that ANF inhibits the release of renin [1, 9, 16]. In addition, ANF has been observed to antagonize the vasoconstrictor actions of angiotensin II *in vitro* [8,19] as well as pressor responses to angiotensin II *in vivo* [4]. Also the hypotensive actions of ANF have been demonstrated to be markedly enhanced in renin-dependent hypertensive rats [17,18], further suggesting an inhibitory effect of ANF on the pressor actions of the renin-angiotensin system. Recently, ANF has been localized in various regions of the brain [7, 11, 15], suggesting that the atrial peptides might also modulate the central actions of angiotensin II. Indeed, recent reports have indicated that central bolus administration of ANF attenuated the dipsogenic actions of angiotensin II [19].

The purpose of the present study was to examine further the central effects of ANF in conscious rats. Drinking responses stimulated by the central injections of angiotensin II were examined during continuous central or peripheral infusions of human ANF (Wy-47,633) to assess the central interactions between ANF and angiotensin II.

METHOD

Male Sprague-Dawley rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and also received presurgical injections of atropine sulfate (0.02 mg/kg, IP) and penicillin (300,000 U, IM). In one group of rats, a single lateral ventricular cannula was stereotaxically positioned in the brain to allow the central administration of compounds. This procedure involved lowering a 23-gauge stainless steel guide tube 0.8 mm posterior to bregma, 1.1 mm lateral to midline and 5.7 mm ventral to the surface of the skull. The cannula was cemented to the skull and sealed with a 30-gauge obturator. In a second group of rats, a second lateral cerebroventricular cannula was inserted opposite the first cannula using the same stereotaxic coordinates. The second cannula facilitated the simultaneous infusion of Wy-47,633 and injection of angiotensin II into the brain. Polyethylene cannulae (P.E. 10) were inserted into the femoral artery and vein to allow measurement of mean arterial pressure and facilitate the intravenous infusion of drugs, respectively.

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Cannulae were tunnelled subcutaneously and exteriorized in the midscapular region. Cannulae were filled with saline and sealed when not in use. Rats were allowed to recover from surgery for a minimum of four days before drinking responses were examined.

All experiments were carried out with conscious unrestrained rats in their home cage environment. Three experimental protocols were followed. In one group of rats, after baseline measurements of arterial pressure and heart rate were recorded, Wy-47,663 [hANF (4–28; 100 ng)] or vehicle (0.1% bovine serum albumin (BSA)-Ringers, 4 μl) was injected into the lateral cerebroventricle via a 30-gauge injector. After three minutes, angiotensin II (15 or 30 ng) was injected intracerebroventricularly (ICV). The delay between the injection of angiotensin II and onset of drinking was recorded as was the volume of water consumed during the first 30 minutes after angiotensin II injection.

In rats with dual ICV cannulae, a continuous ICV infusion of Wy-47,663 (60 ng/min) or vehicle (1.12 μl/min) was begun after baseline parameters were recorded and maintained for 60 minutes. After 30 minutes of infusion, angiotensin II (15 ng/kg) was administered via the second ICV cannula. The onset of drinking and total volume consumed over the final 30 minutes of infusion were monitored. Changes in mean arterial pressure and heart rate were also monitored throughout the study.

In the final group of rats, Wy-47,633 (2 μg/kg/min) or vehicle (10 μl/min) were infused intravenously for 60 minutes. After 30 minutes of infusion, angiotensin II (15 ng) was administered centrally and drinking responses were monitored.

Arterial blood samples were collected at the end of the infusion periods in the latter two protocols to determine plasma "ANF-like" immunoreactivity. Blood was collected in prechilled polypropylene tubes containing EDTA (1 mg/ml) and aprotinin (Trasylol; 1000 KIU/ml). After centrifugation, the resulting plasma samples were stored at −20°C until analyzed for ANF activity by radioimmunoassay. The radioimmunoassay was performed using an antibody (anti-hANF 8798) and procedures provided by Peninsula Laboratories (Belmont, CA).

At the end of all experiments, the patency of the ICV cannulae were assessed by the injection of Evan's blue dye (3 μl) centrally and anatomical verification of the spread of the dye throughout the cerebroventricular system.

All data are presented as means±standard errors of the means. Inter-group comparisons were made using a one-way analysis of variance and a Student-Newman-Keuls nonpaired *t*-test. Values were deemed statistically significant if $p<0.05$.

RESULTS

The central injection of Wy-47,663 (100 ng) or vehicle had no overt effects on the behavior of the rats. No change in arterial pressure was observed after the bolus. Vehicle or Wy-47,663 did not stimulate drinking responses in any of the rats tested, whereas injection of 15 or 30 ng of angiotensin II elicited drinking responses in every rat. In vehicle-treated animals, drinking commenced within 20–40 seconds after injection of angiotensin II (Fig. 1). The time required for dinking to commence appeared to be dose-dependent. The rats drank steadily and over the following 30 minutes consumed 7.9±1.0 and 7.6±0.2 ml after central angiotensin II doses of 15 and 30 ng, respectively. In Wy-47,663-treated rats, angiotensin II still elicited drinking responses but they did not commence until 60–70 seconds after the injection. The latency was approximately twice as long as that observed in vehicle-treated rats. However, once drinking began, the water consumption of the ANF-treated rats over 30 minutes was similar to that for the vehicle-treated group.

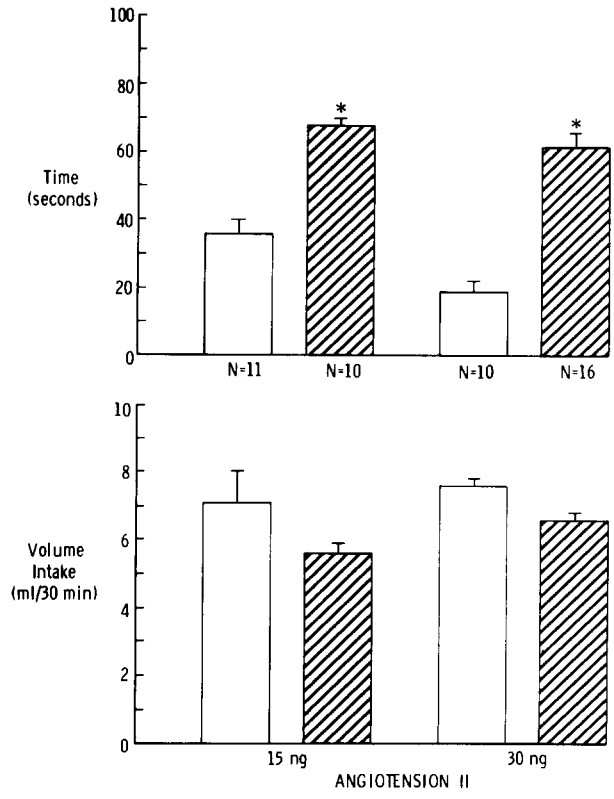

FIG. 1. The effects of centrally administered Wy-47,663 (100 ng; hashed bars) or vehicle (4 μl; open bars) on drinking responses to ICV injections of angiotensin II (15 and 30 ng) in conscious unrestrained rats. The upper graph depicts the time between the injection of angiotensin II and the initiation of drinking. The lower graph illustrates the total water volume consumed over a 30 minute period after injection of angiotensin II. *=$p<0.05$, comparison between vehicle- and Wy-47,663-treated groups.

Central infusion of Wy-47,663 (60 ng/min) or vehicle (1.12 μl/min) had no effect on mean arterial pressure (Table 1). Intracerebroventricular ANF infusion also failed to alter heart rate (373±7 vs. 369±7 beats/min). Again, no gross changes in behavior were observed in the rats and neither ANF nor vehicle infusion stimulated drinking responses. After ICV injection of angiotensin II (15 ng), drinking responses were observed in both groups of rats (Fig. 2). However the initiation of drinking was markedly delayed in ANF-treated rats (205±12 seconds) as compared to vehicle-treated rats (71±8 seconds). Again, ANF- and vehicle-treated rats consumed similar amounts of water over the 30 minute period after angiotensin II injection. Interestingly, there was a significant increase in plasma ANF-like immunoreactivity in the rats administered Wy-47,633 ICV as compared to vehicle-treated animals (Table 1).

Intravenous infusions of Wy-47,633 or vehicle had no effect on arterial pressure (Table 1) or heart rate. Central administration of angiotensin II caused similar drinking responses in both groups of rats. No difference in the time to

TABLE 1

HEMODYNAMIC EFFECTS OF CENTRAL AND PERIPHERAL INFUSIONS OF Wy-47,663 IN CONSCIOUS NORMOTENSIVE RATS

Drug	Baseline MAP	Infusion MAP	Δ	Plasma ANF-like Immuno-reactivity (pg/ml)
Vehicle; ICV (N=6)	119 ± 5	125 ± 8	6 ± 2	692 ± 103
Wy-47,663; ICV (N=17)	110 ± 4	114 ± 3	4 ± 3	1031 ± 97*
Vehicle; IV (N=7)	112 ± 4	110 ± 5	2 ± 3	1037 ± 190
Wy-47,663; IV (N=7)	103 ± 6	102 ± 5	−1 ± 4	17467 ± 3698*

Δ=Change; ICV=intracerebroventricular infusion; IV=intravenous infusion; *=$p<0.05$, comparison to vehicle-treated group; MAP=mean arterial pressure.

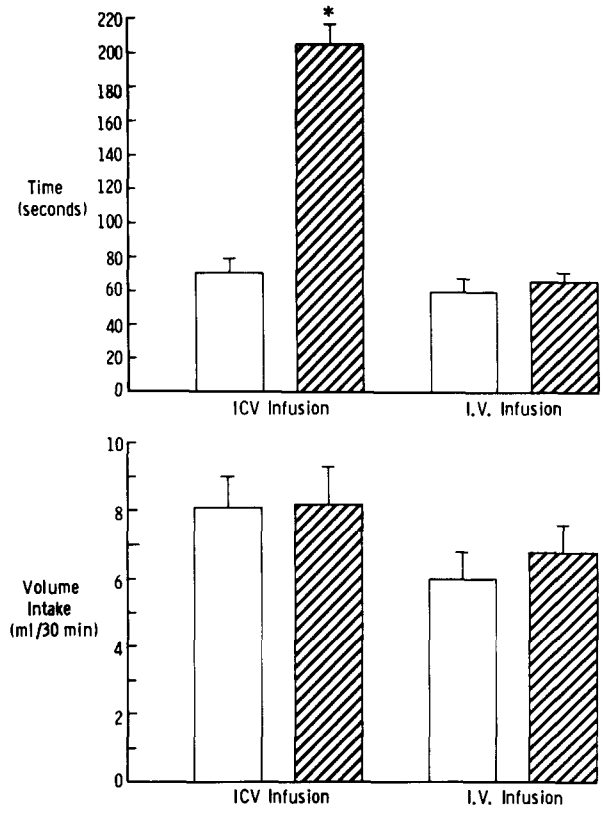

FIG. 2. The effects of central and peripheral infusions of Wy-47,663 (60 ng/min and 2 μg/kg/min, respectively; hashed bars) or vehicle (1.2 μl/min and 10 μl/min; respectively; open bars) on drinking responses to central injections of angiotensin II (15 ng). Upper graph depicts the elasped time between the injection of angiotensin II and the initiation of drinking. The lower graph illustrates the amount of water consumed over a 30 minute period after injection of angiotensin II. *=$p<0.05$, comparison between vehicle- and Wy-47,663-treated groups.

initiate drinking or in the volume consumed were observed between ANF- and vehicle-treated rats, even though plasma ANF-like immunoreactivity was markedly increased in rats infused intravenously with Wy-47,633 (17,467±3698 pg/ml) as compared to rats infused with BSA-Ringers (1037±190 pg/ml).

DISCUSSION

The goal of the present study was to examine the effects of Wy-47,663 (human ANF) on the dipsogenic effects of angiotensin II. It has been well established that injection of small amounts of angiotensin II into the lateral cerebroventricles of rats causes a prominent dose-dependent increase in water consumption [5,10]. Since ANF has been demonstrated to antagonize the actions of the renin-angiotensin system in the periphery, the possibility that the atrial peptides might also attenuate the central effects of angiotensin II was considered. Indeed, injection or infusion of low doses of Wy-47,633 in the lateral ventricles prior to the central injection of angiotensin II markedly delayed the onset of drinking, indicating that ANF could suppress but not abolish the dipsogenic effects of angiotensin II. These findings, coupled with the identification and localization of ANF in the central nervous system [7, 11, 15], suggest that the atrial peptides may modulate extracellular fluid volume by suppressing fluid intake as well as by increasing urinary excretion of salt and water.

Other investigators have also observed the inhibitory effects of ANF on angiotensin II-induced drinking in rats [12]. In those studies, angiotensin II and increasing doses of ANF were administered centrally at the same time. Significant reductions in fluid consumption over 30 minutes were observed with the highest doses of ANF. These results differ somewhat from those reported in the present study, where the drinking responses after angiotensin II were delayed, but total fluid intake was not altered by central ANF. The reason for these discrepancies may be attributable to differences in the doses of ANF used in the two studies. The previous investigators observed an attenuation of fluid intake only after large central doses of ANF (2 and 5 μg). These doses, when injected intravenously, produced marked diuretic and natriuretic responses in anesthetized rats (unpublished data). In the present study, doses of Wy-47,663 were intentionally kept at low levels. Only 0.1 μg of Wy-47,663 was injected into the lateral ventricle. This dose, when injected intravenously failed to cause a significant natriuresis. Similarly the 60 ng/min infusion of Wy-47,663 represents the threshold peripheral dose to produce diuretic responses in anesthetized rats. Thus it is quite possible that fluid intake may have been reduced in the present study if higher doses of Wy-47,663 had been tested. However, the fact that the onset of angiotenisin II-induced drinking was suppressed at the low doses of ANF employed in the present study, clearly demonstrated that the atrial peptides are potent modulators of the dipsogenic action of angiotensin II.

In contrast to ICV administration, intravenous infusion of Wy-47,663 failed to alter the angiotensin II-induced drinking responses, even though the dose employed (2 μg/kg/min) represents a maximally effective diuretic dose of Wy-47,663 (unpublished observation) and caused marked elevations in

circulating levels of ANF-like immunoreactivity. These findings may indicate that peripherally administered Wy-47,663, a 25 amino acid peptide, does not readily cross the blood-brain barrier in adequate amounts to antagonize the dipsogenic actions of central angiotensin II. This is not to say that circulating ANF could not suppress the central effects of blood-borne angiotensin II. Blood-borne angiotensin II has been demonstrated to also initiate drinking responses [5]. In addition, chronic administration of ANF was observed to reduce fluid intake in two kidney-one clip hypertensive rats [6], a high renin model of high blood pressure. These data would suggest that circulating ANF may modulate dipsogenic responses to circulating angiotensin II.

Interestingly, the central administration of Wy-47,663 had no effect on baseline arterial pressure or heart rate in the conscious normotensive rats. It is well established that intravenous administration of ANF in anesthetized and conscious animals generally reduces arterial pressure [1, 9, 13, 16]. With the discovery of ANF in regions of the central nervous system that are involed in regulation of cardiovascular function [7, 11, 15], it was speculated that the peptides may also alter blood pressure through a central mechanism of action. While no evidence of a centrally mediated change in mean arterial pressure was observed in the present study, this is hardly sufficient to conclude that ANF does not have effects on the central nervous system. In fact alterations in nerve activity have been observed after central administration of ANF (personal observation). This may indicate that ANF is involved in reflex control of the circulation or vasopressin release [14]. Further studies are necessary to define the central actions of ANF.

In summary, the central administration of Wy-47,663 delayed drinking responses to central angiotensin II. Similar effects were not observed with peripheral administration of the atrial peptide. These data suggest that ANF may participate in fluid volume regulation by modulating fluid intake as well as through its diuretic actions.

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