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THE RENAL AND VASCULAR EFFECTS OF CENTRAL ANGIOTENSIN II AND ATRIAL NATRIURETIC FACTOR IN THE ANAESTHETIZED RAT

BY K. A. AL-BARAZANJI AND R. J. BALMENT

From the Department of Physiological Sciences, University of Manchester, Manchester M13 9PT

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SUMMARY

1. The interaction between atrial natriuretic factor (ANF) and angiotensin II (Ang II) within the brain to influence renal function and blood pressure was studied in Inactin-anaesthetized male Sprague–Dawley rats.

2. Central infusion of ANF produced a diuresis which was associated with a significant decrease in plasma arginine vasopressin (AVP) level. There was no change in sodium excretion rate over the 80 min of intracerebroventricular ANF infusion and ANF produced no detectable change in mean arterial blood pressure.

3. Central Ang II administration produced a significant decrease in urine flow, which was associated with elevated plasma AVP, an increase in sodium excretion and a rise in mean arterial blood pressure.

4. Combined ANF and Ang II infusion produced an antidiuresis, which was associated with increased plasma AVP concentration. Both the natriuretic and vasopressor actions of central Ang II were abolished when ANF was co-administered.

5. It is concluded that ANF and Ang II interact centrally; ANF antagonizes the pressor and natriuretic effects but not the antidiuretic effects of central Ang II. These data suggest the possibility of distinct and separate sites within the brain through which Ang II influences vasopressin release and renal sodium handling and elevates blood pressure.

INTRODUCTION

Circulating atrial natriuretic factor (ANF), which is synthesized in and released from the atrium, is a peptide with potent diuretic, natriuretic and vasorelaxant properties (de Bold, Borenstein, Veress & Sonnenberg, 1981; Cantin & Genest, 1985; Needleman, 1986). The effects of this peptide oppose the actions of vasopressin and angiotensin II in the kidney, adrenal gland and vasculature (Cantin & Genest, 1985; von Schroeder, Nishimura, McIntosh, Buchan, Wilson & Ledsome, 1985; Lynch, Braas & Snyder, 1986). Indeed, ANF may contribute to the elimination of excess fluid by suppressing renin and vasopressin secretion (Maack, Marrion & Camargo, 1984; Samson, 1985*a*). Immunoreactive ANF neurones and ANF receptors have also been localized and quantified in discrete brain areas of rat and other mammalian species (Cantin & Genest, 1985; Jacobowitz, Skofitsch, Keiser, Eskay & Zamir, 1985; Quirion, Dalpe & Dam, 1986; Saavedra, Correa, Plunkett, Israel, Kurihara &

Shigematsu, 1986). Recently in porcine brain a new twenty-six amino acid residue peptide (brain natriuretic peptide) has been described, which is structurally ANF-like and possesses similar natriuretic, diuretic and vasorelaxant activities (Sudoh, Kangawa, Minamino & Matsuo, 1988). In addition to its peripheral actions, circulating ANF effects on fluid regulation and blood pressure could in part be centrally mediated. Indeed, the highest concentration of ANF in the rat brain are in the hypothalamus and septum, which include the anteroventral third ventricle region (AV3V), areas which are known to be involved in the central control of water and electrolyte balance and blood pressure (Brody & Johnson, 1980; Saper, Standaert, Currie, Schwartz, Geller & Needleman, 1985; Samson, 1985*b*). Intracerebroventricular ANF administration in rats inhibits angiotensin II (Ang II)-induced water intake (Nakmura, Katsuura, Nakao & Imura, 1985), and salt appetite (Fitts, Thunhorst & Simpson, 1985). These central actions of ANF suggest antagonism between ANF and Ang II in the central nervous system as well as in the periphery, and indicates that ANF may act as a neurotransmitter or neuromodulator as well as a circulating hormone.

The present investigation examines the interaction between ANF and Ang II in the brain and its effects on renal function and blood pressure. A preliminary account of some of this work was presented to the 1988 International Symposium on ANP, Kyoto, and to the Society for Endocrinology (1988).

METHODS

Animal

Experiments were performed on male Sprague–Dawley rats (300–350 g body weight) housed in the Animal Unit of the University of Manchester. Animals were maintained on a 12 h light/12 h dark regime and allowed free access to food and water.

Surgical preparation

Animals were anaesthetized with Inactin (110 mg/kg, Byk Gulden, I.P.). Following a tracheotomy, the right jugular vein (Portex PP50) and urinary bladder (Portex PP90) were cannulated. Mean arterial blood pressure (MBP) was monitored continuously via a femoral artery cannula (Portex PP50). A further 23 gauge stainless-steel cannula was located stereotaxically into the right lateral ventricle (co-ordinates: lateral 1·3 mm, anterior 5·9 mm with respect to lambda, and vertical 3·4 mm from the skull surface). Stainless-steel jewellery screws and dental acrylic cement served to secure the cannula in place. Correct placement of the cannula in the lateral brain ventricle was verified by an intracerebroventricular (I.C.V.) injection of 1 % Methylene Blue after the animal had been killed.

Experimental protocol

All animals were placed on continuous I.V. hypotonic saline infusion (0·077 M NaCl, 150 μl/min, Sage Pump Model 351). After a 3 h equilibration period steady rates of urine flow and sodium excretion were established. Three 20 min control urine collections were then made while all animals received I.C.V. vehicle (isotonic saline, 0·154 M-NaCl, 0·8 μl/min, Harvard Pump Type B). For 80 min animals were then divided into four groups to receive either I.C.V. vehicle, Ang II (0·09 ng/min, [Val^5]angiotensin II, Sigma), rat ANF (rANF, 0·4 ng/min; 28 aa Penninsula) or combined Ang II and rANF. All animals were finally returned to I.C.V. vehicle infusate for the remainder of the experimental period. Rates of urine flow and sodium excretion were determined from 20 min urine collections over the 4 h experimental period. Body temperature was maintained at 37 ± 1 °C by means of a heated table. Urine flow rate was determined gravimetrically and urinary sodium concentration by flame photometry (Corning Model 455).

Blood samples were collected from groups of animals prepared in parallel to those for renal study,

but killed at the end of the period of hormone infusion. All samples were taken by trunk blood collection after decapitation (6–8 ml approx.) into chilled plastic tubes containing 100 μl Pepstatin (0·5 mM) and 100 μl EDTA (125 mM). The separated plasma was stored at −20 °C. Following the Sep-Pak (Millipore, Waters Associates) extraction, plasma rANF was determined using a commercial rANF radioimmunoassay (Amersham International) and plasma vasopressin was measured by radioimmunoassay according to the method described by Ashton & Balment (1989). The extraction efficiency for rANF was 83 ± 1·8% ($n = 6$) and for vasopressin was 58 ± 1·6% ($n = 10$); no correction has been made for sample measurements. The intra-assay coefficient of variation for ANF was 7·1% ($n = 9$) and for vasopressin was 17·1% ($n = 10$), respectively.

All values are expressed as mean and S.E.M. For renal excretion and blood pressure measures statistical significance of the effect of treatments and comparisons between control and experimental groups were assessed by two- and one-way analysis of variance. Comparisons of measures at equivalent times in control and experimental groups (shown in Figs 1 and 2) were carried out by Dunnett's test. Student's t test was used to compare plasma hormone levels in control and experimental groups.

RESULTS

Plasma hormone levels

Intracerebroventricular administration of rANF for 80 min caused no change in plasma ANF levels by comparison with animals maintained on hormone-free infusate (298·5 ± 20·8 pg/ml *vs.* control 282 ± 20·3 pg/ml, $n = 8$ for each group). Plasma arginine vasopressin (AVP) concentration by comparison with animals maintained on hormone-free I.C.V. infusate (4·1 ± 1·1 pg/ml, $n = 5$), was significantly reduced following I.C.V. rANF (1·4 ± 0·7 pg/ml, $n = 7$, $P < 0·05$) and increased after infusion of either Ang II alone (7·5 ± 1·1 pg/ml, $n = 6$, $P < 0·05$) or combined rANF and Ang II (9·5 ± 1·1 pg/ml, $n = 5$, $P < 0·05$).

Mean arterial blood pressure (MBP)

For animals maintained on hormone-free I.C.V. infusate MBP remained stable throughout the 4 h experimental period ($F1,11 = 0·32$, $P = 0·96$). In the initial control period MBP was comparable in groups destined to receive saline only (115·8 ± 1·0 mmHg), ANF (115·2 ± 1·3 mmHg), Ang II (113·2 ± 1·8 mmHg) or combined ANF and Ang II (115·6 ± 0·7 mmHg). Figure 1 shows the effects of central infusion of rANF, Ang II and combined Ang II and rANF on MBP. Central ANF administration produced no detectable change, while Ang II alone markedly increased MBP (+13·4 mmHg after 1 h) which declined slowly upon cessation of the hormone infusion. This hypertensive effect of Ang II was abolished when administered in combination with rANF.

Renal water and sodium management

During the pre-treatment control period urine flow and sodium excretion rates were comparable in control (126·4 ± 7·1 μl/min and 6·9 ± 1·1 μmol/min, respectively) and experimental groups which were to receive ANF (122·6 ± 10·1 μl/min and 4·7 ± 1·4 μmol/min), Ang II (128·4 ± 10·6 μl/min and 5·8 ± 0·8 μmol/min) or combined ANF and Ang II (134·3 ± 18·2 μl/min and 5·8 ± 0·8 μmol/min). For animals maintained on hormone-free I.C.V. infusate urine flow ($F1,11 = 48·4$, $P = 0·87$) and sodium excretion rates ($F1,11 = 2·6$, $P = 0·07$) remained relatively stable over the experimental period. However, I.C.V. infusion of 0·4 ng rANF/min for 80 min (Fig.

2*A*) produced a diuresis ($F1,18 = 42{\cdot}3$, $P < 0{\cdot}0001$) which was consistent with the observed fall in plasma AVP concentration. Urine flow increased markedly to reach a peak after 60 min, and upon termination of hormone administration flow rate returned to control levels. This diuresis was not associated with a detectable change in sodium excretion ($F1,18 = 0{\cdot}26$, $P = 6{\cdot}22$), but surprisingly a natriuresis developed upon cessation of the hormone administration (see Fig. 2*A*).

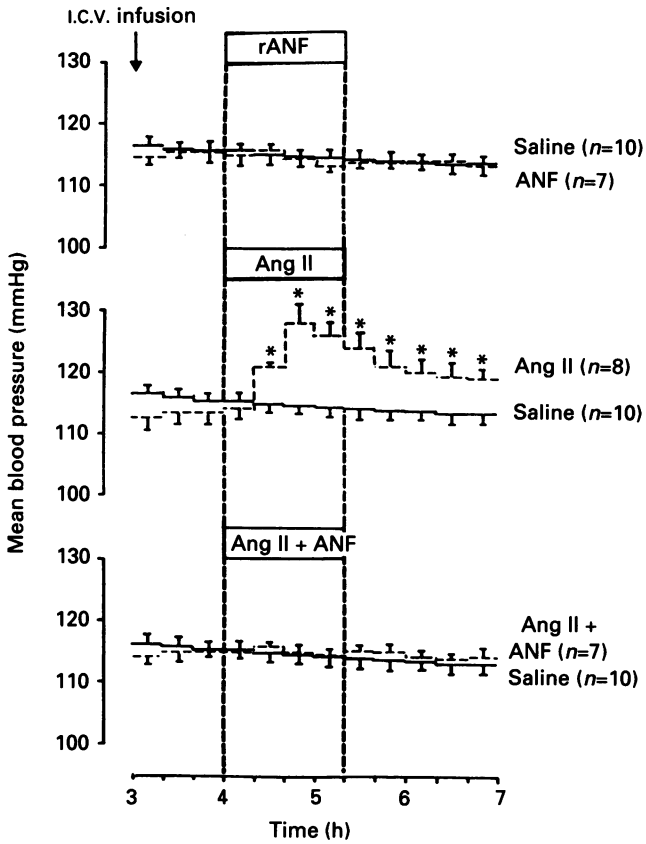

Fig. 1. The effect of I.C.V. infusion of rANF (0·4 ng/min), Ang II (0·09 ng/min) and combined rANF and Ang II on mean arterial blood pressure in anaesthetized Sprague–Dawley rats. Values are presented as means for each 20 min measurement following the 3 h equilibration period; vertical bars indicate S.E. of means. n = number of animals. Statistical comparisons between the vehicle and hormone-treated groups at each time point are by Dunnett's test; $*P < 0{\cdot}05$.

As expected from the observed increase in circulating vasopressin, central infusion of 0·09 ng Ang II/min (Fig. 2*B*) significantly decreased urine flow ($F1,17 = 8{\cdot}05$, $P < 0{\cdot}02$). This was followed by a rebound diuresis ($F1,17 = 15{\cdot}39$, $P < 0{\cdot}01$) upon return to vehicle infusion. A massive increase in sodium excretion ($F1,17 = 23{\cdot}5$, $P < 0{\cdot}001$) accompanied central Ang II infusion (see Fig. 2*B*). This natriuretic effect of Ang II was sustained over the 80 min period of infusion, with sodium excretion returning to control levels upon cessation of hormone administration.

Infusion of combined rANF and Ang II tended to reduce urine output ($F1,15 =$

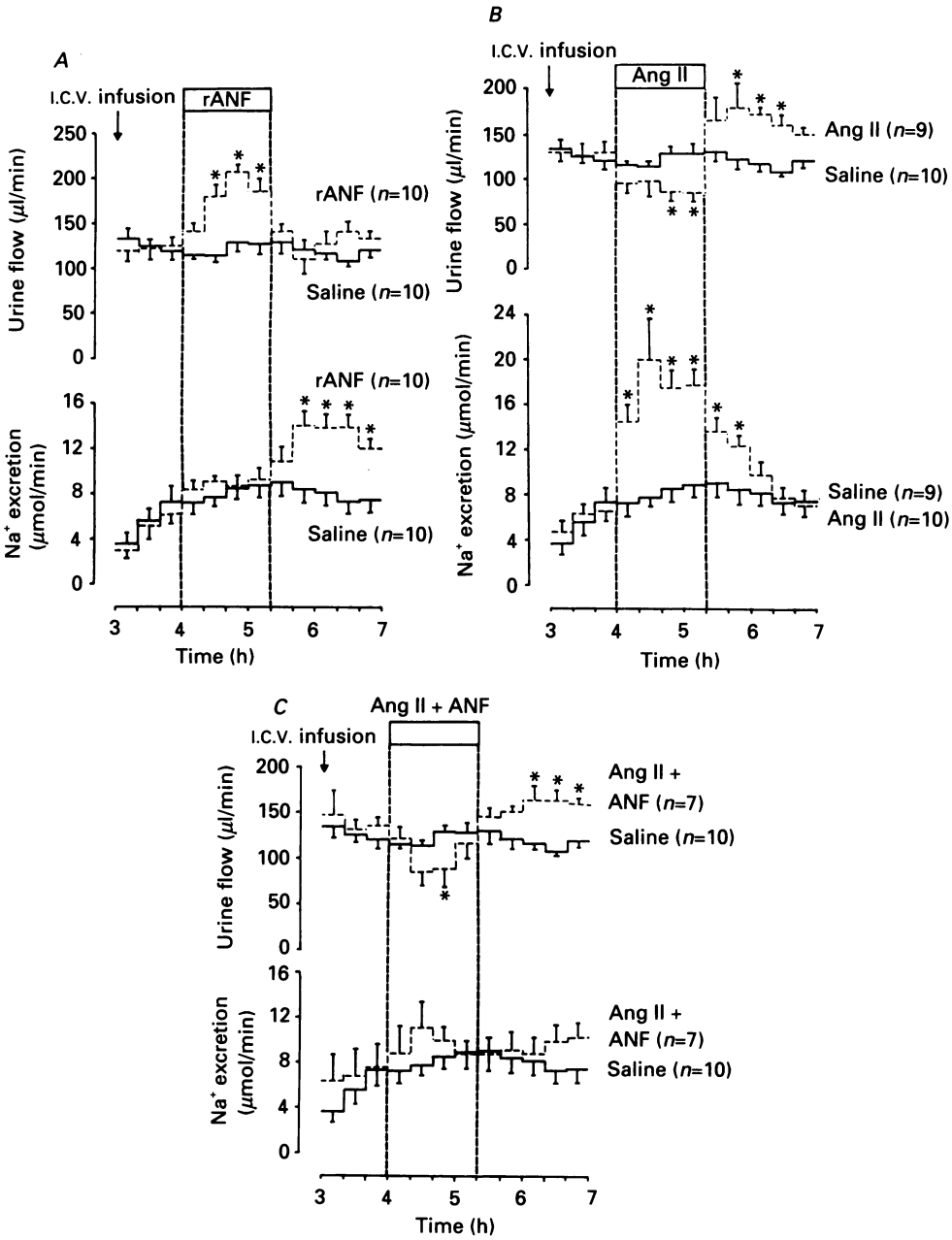

Fig. 2. Urine flow and Na^+ excretion rates in anaesthetized Sprague–Dawley rats infused I.C.V. with: *A*, rANF (0·4 ng/min); *B* Ang II (0·09 ng/min); and *C*, combined rANF and Ang II. Values are presented as means for each 20 min collection period; vertical bars indicate S.E. of means. n = numbers of animals. Statistical comparison at each time point between animals receiving I.C.V. saline and rats receiving the hormone are by Dunnett's test; $*P < 0·05$.

1·61, $P = 0·22$) similar to the effect of Ang II infusion alone (see Fig. 2*B* and *C*). However, this was not associated with a detectable change ($F1,15 = 0·6$, $P = 0·53$) in sodium excretion rates (see Fig. 2*C*).

DISCUSSION

In the present study circulating ANF level was not altered by I.C.V. ANF administration, which suggests that not only was there no peripheral leakage of centrally administered ANF but that there was also no centrally evoked change in the release of systemic ANF.

Central ANF at the dose employed in the present study did not affect basal MBP in agreement with previous observations in conscious, unrestrained rats (Itoh, Nakao, Morii, Yamada, Shiono, Sakamoto, Sugawara, Saito, Katsurra, Shiomi, Eigyo, Matsushita & Imura, 1986) and in the anaesthetized rat and sheep (Lee, Feng, Malvin, Huang & Grekin, 1987). The increment in MBP observed with central Ang II administration is also consistent with previous reports in conscious rats (Hutchinson, Schelling, Mohring & Ganten, 1976*a*, *b*). In these latter studies, AVP-deficient Brattleboro DI animals showed no detectable effect of Ang II on MBP, an observation which led the investigators to suggest that the pressor response to central Ang II was related to systemic AVP release. However, the present work provides evidence that the Ang II-induced rise in MBP could be produced by a direct central Ang II action, possibly independent of AVP secretion, as this hypertensive effect of Ang II but not its effect on neurohypophysial secretion could be abolished or antagonized by ANF. Although the anaesthetized rats received hypotonic saline infusion plasma AVP levels were not depressed, perhaps reflecting stimulation of AVP secretion in response to the stress associated with I.C.V. cannulation. Detectable but lower AVP levels have been reported by us in comparable hypotonic saline-infused preparations of both Sprague–Dawley (Balment, Brimble, Forsling & Musabayane, 1984) and Long–Evans (Forsling, Brimble & Balment, 1982) rats, though these animals lacked I.C.V. cannulae. Plasma AVP concentration was markedly elevated during central Ang II infusion as previously reported in rats and dogs (Keil, Summy-Long & Severs, 1975). The hypertensive effect of central Ang II was suppressed by co-administration of ANF, but plasma AVP levels were still significantly raised in these animals. Thus ANF at the low dose used in the present study was able to antagonize the pressor effect of central Ang II but not its effect on AVP secretion. Similar antagonism by ANF of the pressor effect and the dipsogenic action of central Ang II has been reported in conscious rats (Nakmura *et al.* 1985; Itoh *et al.* 1986). However, those effects were achieved by administration of ANF at doses ranging from 200 ng to 5 μg, which compares with the infusion rate of 0·4 ng/min in the current work.

Modulation of AVP secretion would appear to be a primary mechanism by which central ANF and Ang II influence renal water handling in the present study. The observed diuretic effect of ANF in Sprague-Dawley rats was associated with a significant decrease in circulating AVP. This is consistent with previous reports in conscious rats and sheep (Lee *et al.* 1987) showing that infusion of subpressor doses of AVP prevented the central diuretic action of ANF. The diuretic

action of central ANF differed, however, from that of ANF in the periphery, as this was not associated with a natriuresis (Maack, Camargo, Kleiert, Laragh & Atlas, 1985), as also described in the conscious rat and sheep (Fitts *et al.* 1985; Lee *et al.* 1987). None the less, central administration of human ANF in pentobarbitone-anaesthetized dogs infused I.V.with 0·9% NaCl has been shown to produce a diuresis and natriuresis (Shoji, Kimura, Matsui, Ota, Iitake, Inoue, Yasujima, Abe & Yoshinaga, 1987), a discrepancy which might be due to differences in experimental protocol and/or the molecular structure of the ANF used.

In the present study central Ang II infusion produced the expected increase in circulating AVP level, associated antidiuresis and a sustained natriuresis (Scholkens, Steinbach, Jung, Unger & Ganten, 1983; Unger, Badoer, Ganten, Lang & Rettig, 1988). Thus the peptides Ang II and ANF produced opposing effects on urine flow and vasopressin secretion, which may underlie a delicate interplay between them as part of the central mechanism controlling body fluids. In the periphery there appears to be considerable functional antagonism between circulating ANF and Ang II to influence body fluid management (Burnett, Granger & Opgeworth, 1984; Chartier, Schifferin, Thibault & Garcia, 1984; McMurray & Struthers, 1988). In the current work ANF was infused I.C.V. at rates not sufficient to antagonize Ang II stimulation of AVP secretion. Recent reports, however, indicate that I.C.V. injection of ANF at much higher doses (5 μg) in conscious rats can inhibit the Ang II-stimulated vasopressin secretion (Yamada, Nakao, Morii, Itoh, Shiono, Sakamoto, Sugawara, Saito, Ohno, Kanai, Katsuura, Eigyo, Matsushita & Imura, 1986).

The sustained natriuresis associated with central Ang II infusion was similar to that observed in the anaesthetized dog (Brooks & Malvin, 1982) and the conscious rat (Halperin, Summy-Long, Keil & Severs, 1981). The massive increase in sodium excretion in the present study was associated with a significant rise in plasma AVP concentration and mean blood pressure. None the less, sustained natriuresis can also be induced by I.C.V. injection of Ang II at doses below the pressor threshold in conscious rats, suggesting an action which is independent of systemic blood pressure change (Unger, Rohmeiss & Demmert, 1988). The antagonism of the natriuresis but not the rise in plasma AVP by co-administration of ANF also suggests that this natriuretic action may not be dependent upon the associated stimulation of neurohypophysial hormone secretion.

In summary, the present study demonstrates that ANF and Ang II appear to interact centrally; ANF antagonized the pressor and natriuretic but not the antidiuretic effects of central Ang II nor Ang II stimulation of neurohypophysial AVP secretion. This antagonistic action of ANF on some of the central Ang II effects suggests further the possibility of distinct sites within the brain through which Ang II influences vasopressin release, natriuresis and elevates blood pressure. These sites appear differentially sensitive to the antagonistic action of ANF.

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