Br. J. clin. Pharmac. (1990), **30**, 83S–88S

Atrial natriuretic peptide (ANP) as a neuropeptide: interaction with angiotensin II on volume control and renal sodium handling

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1 Angiotensin II (ANG II) and atrial natriuretic peptide (ANP) are functionally antagonistic circulating hormones involved in blood pressure and body fluid regulation. An inappropriate atrial secretion of ANP has been implicated in the pathogenesis of hypertension, but clinical and experimental results on the role of ANP in hypertension are still conflicting.

2 In the brain both peptides have been localized in close proximity, preferentially in areas involved in central cardiovascular, electrolyte and volume control. ANP was shown to inhibit ANG II-induced drinking, release of pituitary hormones and natriuresis, and to induce sodium retention when given alone.

3 These findings suggest that also in the brain ANG II and ANP exert functionally antagonistic effects. However, in contrast to their peripheral effects, ANG II induces natriuresis while ANP appears to cause antinatriuresis in the brain.

Keywords ANP angiotensin brain rat salt excretion blood pressure

Introduction

Atrial natriuretic peptide (ANP) and angiotensin II (ANG II) are two peptides which have originally been found in the periphery as circulating hormones. Both peptides exert distinct actions on the cardiovascular system and contribute to mechanisms of volume and electrolyte homeostasis.

A wealth of experimental and clinical data lend support to the hypothesis that ANP and ANG II constitute two functionally antagonistic principles with respect to their physiological role in body fluid regulation: ANG II has long been recognized for its sodium and water conserving effects through direct actions on the kidney as well as stimulation of aldosterone release from the adrenal gland and vasopressin release from the pituitary gland. In addition, ANG II can increase blood pressure (BP)—or maintain BP in the case of volume loss—by direct arteriolar vasoconstriction and by facilitating sympathetically mediated vasoconstriction. ANP, on the other hand, was shown to be a very potent endogenous natriuretic agent while at the same time inhibiting the release of renin, aldosterone and vasopressin. In addition, ANP can lower BP by direct vasodilation or, indirectly, by lowering venous return or through vagal mechanisms.

An inappropriately high activity of the renin-angiotensin system (RAS) with its main effector peptide ANG II has been implicated in pathophysiological mechanisms, that can induce or maintain hypertension. In view of the natriuretic and vasodilatory actions of ANP and the obvious

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functional antagonism between ANG II and ANP, it was thought earlier on that an insufficient release or action of ANP might also be one of the factors involved in the pathophysiology of hypertension. However, clinical and experimental results to prove this hypothesis have been rather disappointing. In hypertensive patients and animals circulating ANP was found to be either within normal range or even increased, and there is also no evidence that hypertensives react to exogenous ANP less than normotensives. In view of these findings, Genest *et al.* (1988) have proposed that in hypertension the release of ANP from the cardiac atria may be inappropriate for the elevated BP or increased atrial tension despite apparently normal plasma levels of the peptide. Reviews on this topic have been published (Genest *et al.*, 1988; Lang *et al.*, 1987) and the reader is referred to these articles for detailed information.

ANP and ANG II constitute, however, not only circulating hormones but have also been localized as neuropeptides in the brain. In the following, we shall focus on some of their central actions, which are relevant for cardiovascular and volume regulation. In particular, we shall address the question, whether the functional antagonism between both peptides found in the periphery has correlates in the central nervous system.

ANP in the brain

ANP has been found in the brain, predominantly as the $ANP_{(5-28)}$ and $ANP_{(5-28)}$ peptide, and its binding sites were localized in close vicinity to those of ANG II in periventricular areas, such as the subfornical organ (SFO) and the organum vasculosum laminae terminalis (OVLT) (for review see Imura & Nakao, 1988; Samson, 1987 Standaert *et al.*, 1988). Recently a new peptide of the ANP family has been isolated from brain tissue and termed brain natriuretic peptide (BNP) (Sudoh *et al.*, 1988).

Central cardiovascular effects of ANP

Reports on the central effects of ANP on blood pressure are quite conflicting. Some authors have found no change in blood pressure in normotensive and in hypertensive rats following ANP administration into the brain ventricles (see reviews). On the other hand, large doses of ANP (20 $\mu g\ kg^{-1}$) into the fourth ventricle induced a reduction in blood pressure in hypertensive as well as in normotensive rats. The effect appeared to involve activation of α_2-adrenoceptors since intracerebroventricular yohimbine, an α_2-receptor antagonist, prevented the effect (Levin *et al.*, 1988). Interestingly, the depressor response induced by intracerebroventricular injection of saralasin, an ANG II receptor antagonist, was potentiated by prior central treatment with ANP in spontaneously hypertensive rats (Shimizu *et al.*, 1986). In our laboratory, intracerebroventricular administration of ANP had no effect on blood pressure in conscious or anaesthetized normotensive Wistar rats (unpublished observations) in accord with previous findings (see reviews).

Cardiovascular effects of ANP following injections into localized areas have been reported. Administration of the peptide into the nucleus tractus solitarii where baro- and chemoreceptor afferents terminate induced depressor and bradycardic responses. Injections into surrounding areas were ineffective (McKitrick & Calaresu, 1988). In contrast, when ANP was locally injected into the suprachiasmatic nucleus, an increase in blood pressure was observed which was slow in onset and long lasting (Sills *et al.*, 1985).

Effects of ANP on thirst and sodium balance

It has been reported that spontaneous drinking behaviour can be inhibited, increased or not affected (see reviews) by ANP. However, almost all authors agree that dehydration-induced or ANG II-induced drinking is antagonized by centrally administered ANP. Furthermore, intracerebroventricular administration of ANP antiserum potentiated the water intake induced by intracerebroventricular ANG II or water deprivation (see reviews).

An effect on Na^+ intake has been reported by several authors. In Na^+-deficient rats, central administration of ANP reduces Na^+ intake. Similarly, ANP administered into the cerebral ventricles of spontaneously hypertensive rats was reported to reduce an increased appetite for Na^+.

Central infusion of ANP has been reported to have no effect on Na^+ excretion whilst a single ventricular dose of ANP was reported to elicit an increase in Na^+ excretion independent of mineralocorticoid and neural function (Israel *et al.*, 1988). Similarly, natriuresis was also reported to occur in response to centrally administered ANP in conscious sheep (Parks *et al.*, 1988). In these studies diuresis was observed. We have obtained contrasting results on Na^+ excretion (Unger *et al.*, 1988a). With doses of 1–1000 ng,

intracerebroventricular injections of ANP in conscious rats significantly reduced Na^+ excretion at all doses without affecting urinary flow or blood pressure. In addition, pretreatment with 0.1–100 ng ANP dose-dependently antagonized the natriuretic effect of centrally administered ANG II (Rohmeiss *et al.*, 1989).

Effects of ANP on vasopressin release

Intravenous or intracerebroventricular ANP has been shown to reduce basal circulating levels of arginine-vasopressin (AVP) as well as inhibit the increase in plasma AVP induced by haemorrhage, dehydration or centrally administered ANG II (see reviews).

The site of action for ANP to inhibit AVP release appears to be in the hypothalamus. In rat hypothalamo-neurohypophyseal explants, ANP may inhibit basal release of AVP as well as the release induced by KCl, ANG II and hyperosmotic stimuli. Although in the superfused rat posterior pituitary gland, AVP release has been reported to be reduced by ANP, this has not been observed by others. Further evidence for a hypothalamic site of action is provided by electrophysiological studies. In the paraventricular nucleus of the rat hypothalamus, extracellularly recorded neuronal activity was inhibited in about one-third of the cells tested by pressure injected ANP (see reviews). Similarly, Standaert *et al.* (1987) recently showed a strong inhibitory influence of ANP on presumptive vasopressin-secreting cells in the magnocellular portion of the paraventricular nucleus. Since small volumes were injected and a relatively short latency was observed, these authors concluded that the effects of ANP were mediated by receptors either on the neurosecretory neurons themselves or on adjacent interneurons synapsing onto the secretory neurons. Support for the latter proposal has been obtained by Okuya & Yamashita (1987) who found that ANP could not directly inhibit magnocellular neurons in the paraventricular nucleus.

Angiotensin in the brain

Angiotensin peptides are prominent members of the large family of neuropeptides found in the brain. Extensive reviews on generation, localization and function of brain angiotensin peptides have recently been published (Phillips, 1987; Unger *et al.*, 1988b) and the reader is referred to these articles for detailed information.

Central cardiovascular effects of ANG II

Intraventricularly administered ANG II induces an increase in blood pressure in a number of species including dog, cat, rat and goat. In some species such as the dog and cat, the pressor response is due to activation of the sympathetic nervous system. In the rat an increased release of AVP also plays a role. In hypophysectomized rats, the ANG II-induced pressor response was smaller and there was little increase in blood pressure in homozygous Brattleboro rats.

Since combined treatment with intravenous V_1-AVP receptor antagonists and α_1-receptor antagonists is required to prevent completely the pressor response to intracerebroventricular ANG II in rats, it appears that an increase in the sympathetic nervous system and AVP release act in parallel to elicit the pressor response. However, a generalized activation of the sympathetic nervous system does not occur, since we found that intracerebroventricular doses of 1 to 100 ng ANG II resulted in decreases in efferent neuronal activity in the adrenal, splanchnic and renal nerves and that this inhibition of efferent sympathetic nerve activity was associated with the initial pressure effect of the peptide (Unger *et al.*, 1985). However, if the rats were then allowed to drink, increases in neuronal activity associated with the drinking behaviour were observed.

Areas implicated in the cardiovascular actions of ANG II are the circumventricular organs, the nucleus tractus solitarii and the ventrolateral medulla. In the dog, the area postrema appears equally or even more important. In the rat, area postrema ablations did not affect the pressor response to acute administration of ANG II, but recent evidence suggests that this region may be important in the hypertension induced by chronic ANG II administration (Fink *et al.*, 1987). The circumventricular organs that are the most sensitive to ANG II stimulation are the subfornical organ and the organum vasculosum of the lamina terminalis. Extremely small doses of ANG II injected into either region have been shown to induce a pressor response.

Although a physiological role of central ANG II in blood pressure regulation is still debated, evidence suggests that dysfunction of the brain renin angiotensin system may be involved in the hypertension of spontaneously hypertensive rats (see reviews). This is based on findings in which (i) a reduction of central ANG II function by antagonists or converting enzyme inhibitors induces a depressor response in hypertensive rats and (ii) the levels of ANG II are elevated in spontaneously hypertensive rats and the turn-

over of ANG II appears to be increased in these animals. Furthermore, spontaneously hypertensive rats show exaggerated responses to intracerebroventricular ANG II. Peripheral vascular hypertrophy resulting in increased vascular responsiveness and a dysfunction of the central metabolism of ANG II in the spontaneously hypertensive rats may account for some of these observations.

Effects of ANG II on thirst and sodium balance

ANG II injected into the brain causes dipsogenesis in many species and is effective in very low doses. Since systemically applied ANG II also induces drinking, the circumventricular organs appear to be involved. In addition, the median preoptic nucleus is also an important site. Since this area is inside the blood brain barrier, it seems that ANG II receptors on both the peripheral and central side of the blood brain barrier participate in the ANG II-induced thirst response (for reviews see Mann *et al.*, 1987; Phillips, 1978).

At present it is still difficult to assign a definitive role for ANG II in the physiology of thirst. Evidence to date suggests the peptide may be involved in extracellular dehydration such as is produced by hypovolaemia, however considerable discrepancy between reports exists. These discrepancies almost certainly arise from the complex nature of thirst and the numerous interrelating pathways mediating the response.

Intracerebroventricular ANG II induces Na^+ appetite and altered drinking preference from water to NaCl (Buggy & Fisher, 1974). This occurs with both acute and long-term administration of ANG II (Avrith & Fitzsimons, 1980; Bryant *et al.*, 1980; Buggy & Fisher, 1974). Furthermore, the Na^+ appetite was restored with low doses of ANG II into the third ventricle of rats who had lost, after nephrectomy, their Na^+ appetite in response to peritoneal dialysis. A sensitive area for the ANG II action appears to be the preoptic region. Administration of ANG II, renin or renin-substrate into this region induces an increase in Na^+ intake (Fitzsimons & Wirth, 1978 and reviews).

Central ANG II infusion enhances natriuresis induced by hypertonic NaCl given into the third ventricle or into the carotid artery (Andersson *et al.*, 1972; Olsson & Kolmodin, 1974). More recently, it was found that the natriuretic effect of intracerebroventricular ANG II in the anaesthetized dog occurred without changes in blood pressure, renal blood flow, glomerular filtration rate and plasma aldosterone concentration (Brooks & Malvin, 1982). Furthermore, experiments from our laboratory have shown that extremely low doses of ANG II injected intracerebroventricularly into the conscious rat also increased Na^+ excretion independent of changes in blood pressure and urine flow (Unger *et al.*, 1989). In this study, an indwelling ureteral catheter was used so that changes in urinary Na^+ excretion could be monitored over very short time periods. It was observed that Na^+ excretion began to increase within minutes of the ANG II injection. The response was dose dependent with significant increases in Na^+ excretion occurring with a dose as low as 100 pg, in several cases even 10 pg doses, which both had no effect on blood pressure. As Na^+ intake induced by ANG II is slow in onset, the results suggest that Na^+ loss may be an initiating factor in the ANG II-induced increase in Na^+ intake. However, other factors may come into play later on since rats administered intracerebroventricular ANG II for several hours go into positive sodium balance (Buggy & Fisher, 1974).

Effects on vasopressin release

ANG II given centrally is well known to increase AVP release. The mechanism is unknown at present but may involve direct stimulation of neurons in the supraoptic nuclei (Nicoll & Barker, 1981) or paraventricular nuclei (Harding & Felix, 1987). Indirect effects of the peptide may be mediated through activation of neurons in the subfornical organ projecting to the magnocellular neurons in the paraventricular nuclei via the median preoptic nucleus (Tanaka *et al.*, 1987). Interneurons containing ANG II may be involved since local injection of ANG II into the median preoptic nucleus increased the activity of the same neurons activated by subfornical organ stimulation (Tanaka *et al.*, 1987) and immunocytochemical studies have shown ANG II-IR projections from the subfornical organ to the median preoptic nucleus. Direct ANG II-IR projections from the subfornical organ to the supraoptic and paraventricular nuclei have also been described. These findings may explain the paradox that subfornical lesions prevent the ANG II-induced increase in AVP release *in vivo* but not *in vitro* (Mangiapane *et al.*, 1982; Sladek & Johnson, 1983). Possibly receptors normally only stimulated by endogenous brain ANG II and involved in mediating the AVP response, become accessible to exogenously applied ANG II in *in vitro* preparations.

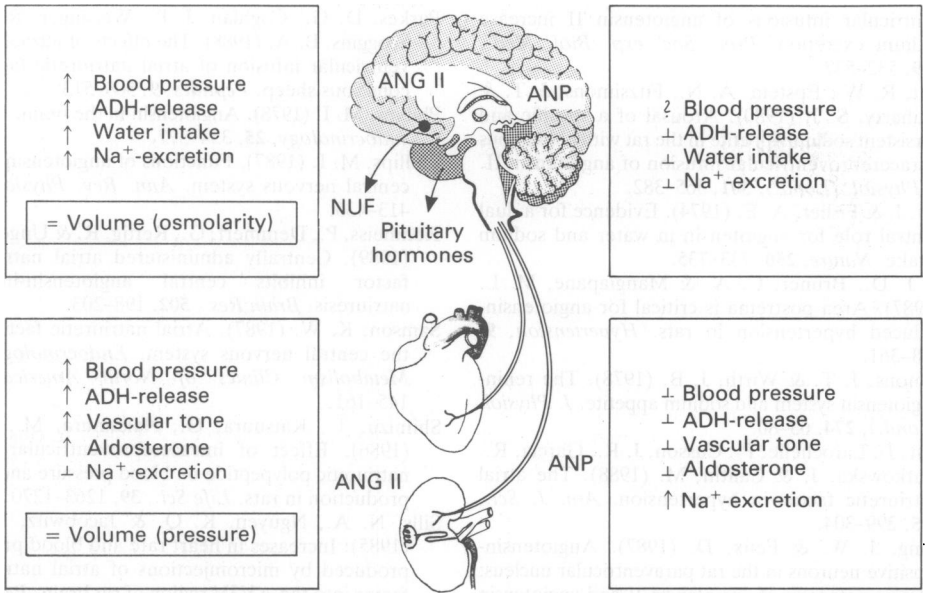

Figure 1 Schematic illustration of the various central and peripheral actions of angiotensin II (ANG II) and atrial natriuretic peptide (ANP). In the periphery, ANG II is involved in volume regulation primarily by its blood pressure increasing and volume retaining actions, while in the brain ANG II may be rather instrumental in osmoregulation (volume preservation and natriuretic effects). ANP, on the other hand, antagonizes most of the peripheral and central actions of ANG II. In contrast to its peripheral natriuretic action, ANP has been shown to induce sodium retention even in the absence of ANG II-induced natriuresis when acting on its receptors in the brain. NUF: a putative hypothalamic Na^+/K^+-ATPase inhibiting natriuretic factor released into the blood. Preliminary data suggest that central ANG II could release such a factor and ANP inhibit its release. Pituitary hormones such as vasopressin. ANG II and ANP act on the release of vasopressin in an antagonistic fashion.

Conclusion

The data presented here demonstrate that both ANP and ANG II can participate as neuropeptides in the central body fluid and cardiovascular regulation. Similar to their opposing actions as circulating hormones both peptides appear to be functionally antagonistic to each other in the brain. Initial thoughts that this central antagonism just repeats the peripheral one on a 'higher level' are not substantiated by the present experimental findings. Thus, in contrast to its peripheral salt and fluid conserving actions, ANG II was shown to be a very potent natriuretic factor when acting on its central periventricular receptors, while ANP, in contrast to its peripheral natriuretic actions, was found, at least in our experiments in rats, to be antinatriuretic and even to prevent the central ANG II-induced natriuresis when acting on its central receptors. Figure 1 gives a schematic illustration of the central and peripheral actions of both peptides documented thus far. Further investigations have to be directed to the physiological relevance of these opposing actions of both neuropeptides on renal salt handling under various conditions and towards the mechanisms involved both on the neuronal and on the renal level.

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