

Twenty Years of Investigating Angiotensin Activity: an Overview

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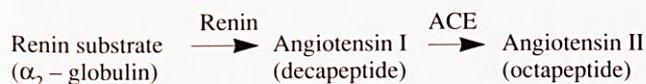
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SUMMARY

A short account of the renin-angiotensin-aldosterone system is given with emphasis on the relationship between the octapeptide angiotensin II and its decapeptide precursor angiotensin I. Although the decapeptide is generally assumed to have no biological activity, extensive investigations have indicated that such an assumption is almost certainly unjustified. The implications of the findings are briefly outlined and discussed.

INTRODUCTION

Since the observation of Tigerstedt and Bergman, reported in 1898, that the kidney contains a highly pressor substance that they named renin¹ there has been intense scientific and clinical interest concerning its *in vivo* operation. Renin itself does not raise the blood pressure directly, being an enzyme that acts on its substrate to release the decapeptide angiotensin I by the splitting of a leucyl-leucine bond.^{2,3} The generally assumed active component of the renin-angiotensin (RA) system, the octapeptide angiotensin II, then results from the loss of histidyl-leucine from the carboxy end of angiotensin I by the action of an endopeptidase, angiotensin converting enzyme^{2,3} now generally referred to as ACE. Thus:



With the involvement of aldosterone also (as discussed by Gross, Brunner and Ziegler⁴) the overall system is known as RAA.

THE EMPHASIS ON ANGIOTENSIN II

The almost universal belief that angiotensin II is the *be-all and end-all* of both RA and RAA activity is based on the findings of Skeggs, Kahn and Shumway in the mid '50s (as indicated in 5; reference 33) that, whereas angiotensin II immediately causes profound increases in renal vascular resistance in the isolated perfused dog kidney, by strong contrast angiotensin I has a negligible straightway effect on it. It was therefore concluded that conversion of the deca- to the octa- peptide by a circulating plasma enzyme is necessary for pressor activity within the kidney and presumably at other vascular receptor sites for angiotensin II. The supposed plasma conversion then held sway for some fifteen years until several groups, employing widely differing experimental approaches, showed that ACE activity in the circulating blood could not account for the rapid and efficient *in vivo* generation of angiotensin II. It became evident that the lungs were important for the formation of the octapeptide and its immediate release into the pulmonary vein but that ACE activity was presumably not confined to that organ (as indicated in 5; 4). The intra-pulmonary conversion satisfied almost all investigators in both the RA and RAA fields since it did not disturb their cherished conviction that angiotensin II was omnipotent, a belief no doubt buttressed by its more ready availability than that of angiotensin I. However the shift of emphasis from plasma to tissue convertibility was profoundly to affect ideas concerning the ability of drugs to modify RA (and RAA) operation by the inhibition of ACE activity at tissue level, resulting in blood pressure reduction.

THE EMERGENCE OF ANGIOTENSIN I

Until 1970 synthetic angiotensin I was difficult to obtain although by then a few studies with its use had been reported. The subsequent greater availability of it tended to confirm the general conviction that angiotensin II is *the* active component of the RA system. However strong doubts were expressed in some quarters; these especially concerned the nature of pressor responses established in anaesthetised sheep⁶ and those in conscious rabbits observed by Munday, Noble and Rowe at Southampton University (refer 5; 28-31) and also the suggestion that angiotensin I itself could have an antidiuretic effect on the peritubular capillaries that is plasma [NaCl] dependent.⁷

ANGIOTENSIN RECEPTOR DISTRIBUTION

The importance of the pulmonary circulation in the conversion of angiotensin I to angiotensin II has been established in extensive studies.^{6,8} These and many other investigations (refer 5; 1-3, 5, 7) have highlighted the strikingly contrasting manner whereby the two peptides are handled by the lungs. Thus an Acceptance/Rejection (AR) situation occurs in that organ in which there is a highly effective removal of the decapeptide from the bloodstream but with free traverse through the pulmonary circulation for the octapeptide itself. However the possibility that differential treatment of the angiotensins happens elsewhere in the body has not received a great deal of attention. That the peptides have complex relationships within the kidney has been argued recently^{5,9} a matter that concerns the question whether common receptors invariably occur in the renal vasculature – ie an Acceptance/Acceptance (AA) situation throughout – or is there involvement of AR and/or RA receptor sites?

The pulmonary generation of angiotensin II clearly ensures a widespread distribution of the octapeptide throughout the systemic circulation. It is therefore not surprising that intravenous injections of angiotensins I and II produced almost identical effects concerning increases in blood pressure⁶ reductions in renal blood flow – RBF¹⁰ and water shifts into renal venous blood.^{11,12} However, with injection into the left ventricle (LV) – although the pressor responses to the angiotensins are not too dissimilar – the immediate ability of the decapeptide to lower RBF¹⁰ and to increase the relative plasma content of blood draining from the kidney^{11,12} is very much less than that of the octapeptide.

The abilities of the intravenously injected peptides to produce such similar effects could well be considered to support the belief that *all* biological effects attributable to renin-angiotensin operation reside in angiotensin II. However it is not possible to reconcile such a viewpoint with the observed renal venous plasma [creatinine] changes that occur with injection of the angiotensins into the general circulation. Thus, whereas with intravenous injections of angiotensin II (and also with injection into the LV, ie with direct presentation to the kidney) the [creatinine] of renal venous plasma is greatly raised, intravenously injected angiotensin I invariably considerably lowers the plasma creatinine levels.^{5,9} There is therefore the paradoxical situation that, although with intravenous administration both peptides similarly affect blood pressure, RBF and the relative plasma content of blood from the renal vein, their effects on the creatinine content of blood leaving the kidney are diametrically opposed. The explanation probably involves a complex interplay between angiotensins I and II in the kidney that importantly concerns the question of the distribution of intra-renal angiotensin receptors and also that of blood pressure and blood and water flows.⁵ These ideas are highly pertinent to the question of acute renal failure precipitated in patients by ACE inhibition; this is a not uncommon effect of such treatment.^{13,14}

Because arterial plasma renin and angiotensin I levels are invariably much increased by ACE inhibition – with a concomitant lowering of angiotensin II concentrations (refer 5;

80–82) the questions of adrenal receptors for the peptides is currently of great interest. With infusions over several hours into the adrenal arteries of conscious sheep¹⁵ the decapeptide was found, on average, to be very much less effective than was the octapeptide in stimulating the zona glomerulosa to secrete aldosterone. When considered together with a later report that, *in vitro*, bovine adrenal gland receptors showed “low or negligible binding of angiotensin II precursors such as angiotensin I and the tetradecapeptide sequence of renin substrate...”¹⁶ there is clearly a strong possibility that a RA situation exists in the adrenals.

The probable existence of an AA situation in the hind-limbs has been indicated by extensive studies utilizing angiotensins I and II and a variety of experimental techniques. This has enabled one aspect of the relationship between the peptides to be closely examined, especially concerning the effects of ACE inhibition (as discussed in 5; 39, 86). However, despite the ability of the angiotensins to lower hind-limb blood flow with direct presentation to the limbs – the decapeptide is mostly reckoned to be 10 to 40% as potent as the octapeptide – large increases in flow can readily be achieved with injection of either peptide into the general circulation (refer 5; 23) indicating the negligible significance of local vasoconstriction.

ANGIOTENSIN – PRODUCED ARTERIAL PLASMA [K⁺] RISES AND [Na⁺], [Cl⁻] FALLS

The ability of angiotensin II simultaneously to raise the circulating plasma [K⁺] and to lower the levels of the sodium and chloride ions was initially reported by Healy, Elliott and Harrison¹⁷ who employed one hourly intravenous infusions. A similar pattern of arterial plasma [electrolyte] response was also evident with 6 minute intravenous administration of the octapeptide^{18, 19} and also with that of the decapeptide;^{9, 18} the pressor catecholamine noradrenaline, however, only minimally released potassium ions into the circulation and did not affect the plasma levels of the sodium and chloride ions.¹⁸ These various observations importantly relate to the well documented influence of Na⁺, K⁺ concentration ratios in influencing aldosterone secretion¹⁵ and the relative inability of noradrenaline – in comparison with the effects of angiotensin II – to enhance the release of the steroid from the adrenal cortex.^{15, 20} The findings with use of the angiotensins were surprising since, until the report of Healy and his colleagues, it was almost universally considered that they had little, if any, ability to influence arterial plasma electrolyte concentrations. This is particularly so because the liberated potassium was readily detectable at infusion rates of only 0.5 to 2µg per minute^{17, 18, 19}

Further reports concerned the use of the released K⁺ ions as a possible marker for the intra-cellular transfer of Ca²⁺ ions²¹ and the question of lung involvement respecting the observed pattern of plasma [K⁺] increases²² (when it was concluded that the most likely source of the potassium was the resistance arterioles of the systemic circulation). Another interesting aspect involves the possibility that the additional circulating potassium ions could have a vasodilator effect.²³ The importance of establishing the cellular electrolyte distribution changes associated with the use of highly pressor compounds requires no emphasis because of the involvement of such variations in the genesis and maintenance of hypertension.²⁴

CONCLUSIONS

The findings of Departmental investigations during the past 20 years – that concerned the effects of angiotensins I and II on (a) blood pressure (b) RBF and intra-renal water, electrolyte, creatinine and urea distribution and creatinine elimination from arterial plasma (c) hind-limb blood flow and (d) arterial plasma electrolyte levels – have extensive physiological and clinical implications. They do not support the almost universal conviction that the effects of RA, and RAA, operation are mediated solely through angiotensin II but strongly indicate a vital role for its precursor angiotensin I in affecting blood pressure in a volume-related manner that is [NaCl] dependent and mediated *via* the peritubular capillaries.

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RESULTS

From a total of 740 patients in three hospitals, 59 (8%) were diagnosed as having Down's syndrome. Their ages ranged from 23 to 65 years (average age 48.3 years) and they were all moderately or severely mentally handicapped. The sexes were evenly divided.

Chromosomal analysis was performed on 48 patients; the remainder were either uncooperative to venepuncture or died or were discharged before it could be performed. Out of the 48, 44 had trisomy 21, one a translocation and three were mosaics.

Radiological examination was possible in 48 patients. Of these three were found to be in the high risk group (atlanto-odontoid distance >5 mm). Five were in the medium risk group (atlanto-odontoid distance 3–5 mm) and 39 were low risk (atlanto-odontoid distance <3 mm). (Table 1).

Table 1

	Results of radiological examination			Total
	Low risk (<3 mm)	Medium risk (3.5 mm)	High risk (>5 mm)	
Males	18	3	3	24
Females	22	2	0	24
Total	40	5	3	48

Neurological examination was performed on 49 patients, and each was given a score according to the five parameters developed by Alvarez and Rubin (Fig. 2). 11 patients scored two or more, which put them into the high risk category, 21 were medium risk, and 17 low risk. (Table 2).

Table 2

	Results of neurological examination			Total
	Low risk (0)	Medium risk (1)	High risk (2+)	
Males	9	9	5	23
Females	8	12	6	26
Total	17	21	11	49

When the results were correlated, it was found that of the three patients in the high risk category radiologically, two were in the medium risk group on neurological examination and one low risk. Of the five patients in the medium risk group on X-ray, four were in the medium risk group neurologically (one was uncooperative to neurological examination). Therefore of those patients with an atlanto-odontoid distance greater than 3 mm, six (75%) had evidence of neurological damage. A total of eight patients (13.6%) could be considered to be at risk of atlanto-axial instability from radiological evidence, though none of them are currently in the high risk group on neurological examination. There was a significant preponderance of males affected (male: female—3:1), and the three high risk patients were all male. Of the 11 patients who had a high score on neurological examination, none of them had evidence of atlanto-axial instability on X-ray.

DISCUSSION

Conflicting opinions have been expressed regarding both the prevalence of atlanto-axial instability in Down's syndrome and the likelihood of it leading to permanent neurological damage. In a recent review of the subject in 1987, Collacott³ concluded that the prevalence appeared to be between 12% and 22%. The current study gives an overall prevalence of 13.6% which concurs with previous findings. However, the prevalence of actual cervical cord damage had been thought to be in the region of 2.3%⁴ and to occur only when the atlanto-odontoid interval is greater than 7 mm. This study has shown that careful neurological examination may uncover hitherto unrecognised pathology, with 10.2% showing some evidence of neurological damage.

However, there are difficulties in interpreting the results, due to other factors involved. For example, none of the

patients in the high risk category neurologically had radiological evidence of atlanto-axial instability so their neurological damage must have had other causes. Six of them had evidence of cervical spondylosis, and one had had a previous cardio-vascular accident. This demonstrates the necessity for radiological screening, as one cannot rely on clinical examination alone in patients who may have multiple pathology.

Although the numbers involved are small, the preponderance of males affected is significant, and has not been noted in previous studies. Indeed, the opposite has been the case⁴. The reasons for this are not known.

An interesting finding not directly relevant to the study is the high prevalence of cervical spondylosis and other degenerative changes uncovered. This occurred in patients in the 40–65 age range, with one patient aged 37 involved, thus providing more evidence of the premature ageing process in people with Down's syndrome.

People with Down's syndrome, in common with all those with a mental handicap, now have many more opportunities for leading as 'normal' a life as possible. This may mean they are participating in activities hitherto not open to them e.g. some sports, that they travel a lot more than previously, and that they may be more likely to require anaesthesia for operations. All these activities may lead to cervical cord damage in a person with undiagnosed atlanto-axial instability. Although some degree of risk taking may be unavoidable, even necessary, it seems that the problem of atlanto-axial instability is one area in which we can minimise the risk by screening. The findings in this study support the necessity for this.

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