



RADIO-IMMUNOASSAY OF ANGIOTENSIN II

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CHAPTER 1

INTRODUCTION

1. RENIN-ANGIOTENSIN SYSTEM

(i) HISTORICAL

The existence of the renin-angiotensin system was first suspected following the work of Tigerstedt and Bergman (1898), who demonstrated a prolonged rise in arterial blood pressure in anaesthetized rabbits following the injection of extracts of rabbit kidney. The renal pressor substance was found in saline extracts of fresh rabbit kidney or of the dry residue obtained after treatment of rabbit kidney with alcohol. The authors coined the name "renin" for the active substance, which was obtained from cortex but not to any appreciable extent from medulla of the kidney. The main properties of the substance were: it was non-dialyzable, stable at 56°C but destroyed by boiling, soluble in water, glycerine and in dilute salt solutions, but insoluble in acetone and in alcohol. The blood pressure rise was not abolished by section of the cervical spinal cord nor by destruction of the spinal cord, indicating that the effect of the pressor agent was independent of the nervous system.

The classic paper of Goldblatt et al (1934) described the production of sustained hypertension in dogs by renal artery constriction with a clip. Constriction of one renal artery led to a slight to moderate rise in blood pressure with a tendency to return towards normal after a period of time, whereas bilateral renal ischaemia commonly produced very high levels of blood pressure.

The authors felt that the hypertension produced by only moderately severe renal artery constriction, with no signs of significant renal functional impairment, resembled benign nephrosclerosis in man, whereas severe constriction from the beginning led to marked elevation of blood pressure, severe disturbance of renal function, and uraemia, the latter picture resembling malignant nephrosclerosis in man. The production of experimental hypertension with a resemblance to clinical varieties of hypertensive disease in man stimulated a large amount of work aimed at defining the role of the kidneys in this situation.

The work of Tigerstedt and Bergman was confirmed in 1938 by Pickering and Prinzmetal and by Landis et al. Pickering and Prinzmetal showed that a prolonged rise in blood pressure may be produced in the anaesthetized rabbit by intravenous injection of extracts prepared from fresh kidney of several species. They confirmed that the active substance, "renin", was present in the cortex but not in the medulla of the kidney and that it had chemical properties suggestive of a protein structure. They showed also that the substance could be assayed biologically by comparison of the blood pressure response produced in unanaesthetized rabbits by the extract with that produced by a standard preparation of renin. Landis et al (1938), working with rabbits, found that "unheated kidney extracts" had a variable effect on blood pressure but that heating to 55-56°C for 20 minutes and filtration produced an extract which consistently elevated blood pressure. The substance responsible for the pressor

activity of renal extracts was destroyed by heating to 65°C, did not pass through a dialysis membrane, and was precipitated with ammonium sulphate.

Goldblatt (1938) and Wilson and Pickering (1938) described the experimental production in animals, by renal artery constriction, of the fibrinoid arteriolar necrosis characteristic of malignant hypertension, in the same distribution as in man except for the sparing of the kidney. The sparing of the kidney prompted the suggestion (Goldblatt, 1938) that the height of the arterial pressure was an important factor in the production of the arteriolar lesions.

In 1939, Page, and Braun-Menéndez et al independently showed that renin had the properties of an enzyme, splitting a plasma constituent into a smaller molecule now known as angiotensin. Page found that injection of purified renin in Ringer's solution did not produce vasoconstriction in experimental animals, but that incubation with "renin-activator" in plasma restored the vasoconstrictor activity. This type of observation led to the concept of renin acting on a plasma substrate to form an active vasoconstrictor substance.

The existence of two forms of angiotensin was demonstrated by Skeggs et al (1954). They showed that the first form, angiotensin I, was formed by the enzymatic action of renin on a plasma substrate and that this was rapidly converted to angiotensin II by the action of a second enzyme present in plasma.

The biochemical definition of the renin-angiotensin system was taken further by Lentz et al (1956). They showed that angiotensin I contained one mole of leucine and one mole of histidine in addition to

the amino acids of angiotensin II, and that conversion of angiotensin I by converting enzyme involved hydrolysis of the phenylalanine-histidine bond to form angiotensin II and histidyl leucine.

The amino acid sequence for horse angiotensin II was described in 1956 by Skeggs et al, and is as follows: asp-arg-val-tyr-iso-his-pro-phe. The amino acid sequence of angiotensin formed by the action of rabbit renin on ox substrate was reported at about the same time (Peart, 1956; Elliott and Peart, 1957); the amino acid sequence was shown to be: asp-arg-val-tyr-val-his-pro-phe-his-leu. It was thought that all the amino acids had the L-configuration and that the N-terminal residue was aspartic acid, not asparagine. The synthesis of angiotensin was achieved shortly afterwards by Bumpus et al (1957) and by Schwyzer et al (1958).

Due to the above work, the concept of the renin-angiotensin system had developed as follows:

