The effect of angiotensin II on the release of catecholamines in the sheep

Numerous investigations have been concerned with a possible relation between the renin-angiotensin system and the secretion of catecholamines from the adrenal medulla and autonomic nerve endings (McGiff & Fasy, 1965; Palm, 1968; Pals, Fulton & Masucci, 1968; Peach & Ford, 1968). The present investigations were concerned with the effect of large intravenous doses of angiotensin II, and of a mixture of adrenaline and noradrenaline, on plasma levels of vanilmandelic acid and on urinary concentrations of the catecholamines and their metabolites.

The angiotensin II was synthetic asparaginyl^{1}-valyl^{5}-angiotensin II (Hypertensin, Ciba); the adrenaline was supplied by Gale Baiss and Co. Ltd., and the noradrenaline acid tartrate (Levophed) by Bayer Products. Saline solutions of the three hormones were made to the required strength and constant infusions (0·7 ml/min from a 50 ml capacity syringe) were made with a slow injection apparatus (C. F. Palmer, London, Ltd.).

A Kerry Hill ewe, 35 kg, was prepared as described by Osborn, Hughes & others (1969). A 20 ml blood sample was taken from the femoral artery 45 min after preparation, and 15 min before the start of an infusion of 2 μg/kg min^{-1} of angiotensin II into the jugular vein. The blood was added to a 25 ml polystyrene bottle, containing 5 ml of an EDTA-thiosulphate solution (Weil-Malherbe, 1961). Similar samples were prepared from femoral artery blood collected 1, 2, 3, 5, 15, 29 and 90 min after the start of the infusion of angiotensin which lasted 30 min. Urine was collected from the bladder *via* an indwelling catheter for 60 min before (Control, Table 1), during, and for 15 min (Angiotensin II infusion, Table 1) after the infusion, then for a further 75 min (Resting, Table 1).

An infusion of a mixture of adrenaline and noradrenaline (2 μg/kg min^{-1} of each) for 30 min was begun 90 min after the end of the infusion of angiotensin. Arterial blood samples were taken 5 min before the end of the infusion of the catecholamines and urine was collected during the infusion and for 15 min afterwards (Catecholamine infusion, Table 1).

The mean resting blood pressure of about 90 mm Hg rose to 145 mm Hg during the initial 10 min of the angiotensin II infusion; thereafter the pressure gradually fell so that it was about 110 mm Hg at the end of the infusion. A similar initial rise resulted from the administration of the catecholamines but it was much better maintained.

Duplicate estimations of vanilmandelic acid were made (O'Gorman, 1968) in plasma prepared from the blood samples by centrifugation at 800 *g* for 10 min at 4°; the plasma was subsequently stored at −20° before analysis. The urine was made approximately 0·2N with concentrated HCl immediately after collection. One-fifth aliquots were transferred to polystyrene bottles and stored at −20°. These were subsequently analysed for vanilmandelic acid (O'Gorman, 1968), and for metadrenaline and normetadrenaline (Gjessing, 1964). Aliquots (40 ml) were analysed for adrenaline, noradrenaline and dopamine by adsorption onto alumina at pH 8·4 using Gout's method as described by Udenfriend (1962) after a preliminary hydrolysis by boiling under reflux for 10 min. Adsorption onto Amberlite columns was at pH 6·1; the subsequent elution was with N HCl. Aliquots of the eluates were analysed by the trihydroxyindole method (von Euler & Lishakjo, 1961) at 3·5 and 6·5 and by the ethylenediamine condensation method (Weil-Malherbe, 1961). The sum of the three catecholamines is given by the condensation method while adrenaline and noradrenaline are estimated by the trihydroxyindole procedure. The difference between the two is the amount of dopamine.

Table 1. *Urinary excretion of catecholamines and metabolites* (*μg/min*). The amounts of urine collected for the successive periods were 114, 90, 88 and 344 ml respectively.

	Control	Angiotensin II infusion	Resting	Catecholamine infusion
Adrenaline	0·01	0·01	0·02	0·75
Noradrenaline	0·09	0·09	0·04	0·51
Metadrenaline	0·05	0·05	0·02	0·15
Normetadrenaline	0·07	0·04	0·02	0·18
Vanilmandelic acid	0·18	1·1	0·18	4·44
Dopamine	0·02	0·06	0·09	0·20

The average level of plasma vanilmandelic acid of 155 ng/100 ml (s.d = ± 10 ng/100 ml) during the infusion of angiotensin II was similar to the level before the infusion (165 ng/100 ml). The plasma sampled immediately before the infusion of the catecholamines contained 190 ng/100 ml; the concentration had risen to 260 ng/100 ml towards the end of this infusion.

The results of the urinary analyses are shown in Table 1. The output of urine was reasonably constant before, during, and after the infusion of angiotensin II. However it was much increased during that of the catecholamines.

The findings show that the infusion of catecholamines caused a rise in plasma vanilmandelic acid and this was reflected in an approximate 25-fold increase in the urinary excretion of this compound. In addition there was a significant increase of several other catecholamine metabolites. By contrast, angiotensin II, which produced a comparable rise of blood pressure, caused no detectable change in plasma vanilmandelic acid; however its infusion was associated with a significant rise in urinary vanilmandelic acid excretion. This is in keeping with previous suggestions that angiotensin II is associated with catecholamine release and our results suggest that measurement of urinary vanilmandelic acid is an acceptable index of this action.

The experiment was made at the Dr. Leonard West Research Laboratory, Sully Hospital, Sully, Glamorgan, Wales, and the expert technical assistance of Mr. J. Wilson is gratefully acknowledged.

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February 26, 1971

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The effect of angiotensin I on renal blood flow in sheep

Injections of angiotensin I or angiotensin II into the renal artery of the dog are immediately effective in reducing renal blood flow (Halvorsen, Fasciolo & Calvo, 1959; Barac, 1962). But Ng & Vane (1967, 1968) found angiotensin I to have no immediate constrictor effect on the renal vessels of the dog, and to require conversion to angiotensin II in the pulmonary circulation to be effective. We now report the action of angiotensin I on renal blood flow in the sheep.

The angiotensin I used was synthetic asparaginyl^{1}-valyl^{5}-angiotensin I (Osborn, Pickens & others, 1970) which is the angiotensin I equivalent of the angiotensin II (Hypertensin). Both hormones were supplied by Ciba Ltd., Basle. When the angiotensin I was tested against the angiotensin II using the rat isolated colon (Regoli & Vane, 1964) in Tyrode solution in twelve experiments, the material was shown to contain less than 1% of angiotensin II.

Five Kerry Hill ewes 35 kg (s.d. = $\pm$ 2 kg) (Osborn, Hughes & others, 1969), had renal blood flow measured (Cohn & Gombos, 1965). Renal vein blood was withdrawn by a 105 A Gilford constant withdrawal pump, through a 103 IR Gilford cuvette densitometer. The dye curves were recorded on a Moseley 710 BM recorder and the areas under the curves estimated after extrapolation of the down stroke. Calibration was effected *in vitro* by adding various amounts of indocyanine green to renal vein blood.

The indocyanine green (0·25 mg in 2 ml saline) was injected into the renal artery over 1 s. As the internal volume of the catheter was 0·2 ml, 1·8 ml of the dye was injected into the artery. Blood was sampled from the renal vein at a constant rate before, during and after the injection of the dye until the extinction of the blood remained constant for 10 s.

The validity of the approach was tested with the angiotensin II in an experiment in which doses of 0·02–1·0 μg of the hormone were injected into the renal artery. Injections in all experiments were made as 5 ml solutions in saline followed immediately by a wash with 2 ml of saline over 2 s. Each injection of the hormone was followed 5 min later by 7 ml of saline given as a divided dose of 5 ml (over 5 s) and 2 ml (over 2 s).

The duration of the effect of 0·20 μg of angiotensin II was estimated by injecting the indocyanine green 6, 10, 15, 20, 30, 45 s and 1, 2 and 3 min after the end of the injection of the hormone. This dose and these times were chosen since about 10% of the cardiac output flows through each kidney and because previous studies in sheep had shown that 2 μg of angiotensin II injected into the left ventricle usually increased the blood pressure by 15–20 mm Hg, the pressor effect lasting for about 3 min. The results demonstrated that angiotensin II caused a significant reduction in renal blood flow within 10 s of the conclusion of the injection of the hormone; the effect was maximal at + 15 s and was well maintained for a further 30 s. Thereafter it gradually declined so that at + 3 min the flow had returned to normal.

These results indicated that a suitable time at which to inject the indocyanine green was 20 s after the administration of the hormone; further experiments were therefore made in the same animal in which the dye was injected 20 s after doses of 0·02, 0·05,