Pressor responses in sheep to angiotensin II, noradrenaline and tyramine

Previous reports have dealt with a possible relation between the actions of angiotensin II, noradrenaline and tyramine and their effects on the autonomic nervous system (McCubbin & Page, 1963a,b; Louis & Doyle, 1966; Scroop & Walsh, 1968; Day & Owen, 1969). We have examined the pressor effects of the three compounds after intravenous injections in sheep. Fourteen Kerry Hill and Welsh Mountain ewes and wethers of average weight 36 kg (s.d. = $\pm$4 kg) were used. The general preparation and recording of blood pressure were according to Osborn, Hughes & others (1969). The angiotensin II was asparaginyl^1-valyl^5-angiotensin II (*Hypertensin*, Ciba), the noradrenaline was noradrenaline acid tartrate (*Levophed*, Bayer Products) and tyramine hydrochloride was supplied by British Drug Houses. Injections were made into the jugular vein as 5 ml solutions followed by a wash with 2 ml of saline over 2 s.

Doses of angiotensin II (10 μg) and of noradrenaline (20 μg) which, by themselves, raised the blood pressure by 25–30 mm Hg, or mixtures of these doses, were injected in the sequence angiotensin II, noradrenaline, angiotensin II *plus* noradrenaline. Three series of injections were made in each of the twelve experiments, the injections being made at 6 min intervals. The average maximum rise of 28 mm Hg with either hormone alone was increased to 45 mm Hg when the two were mixed while doubling the dose of angiotensin II from 10 to 20 μg and of noradrenaline from 20 to 40 μg only raised the blood pressure to 32 mm Hg.

The effect of sequential injection of angiotensin and noradrenaline was investigated by injecting 20 μg of noradrenaline 4 and 10 min after 10 μg of angiotensin II, and also in the reverse order by giving 10 μg of angiotensin II 2 and 8 min after 20 μg of noradrenaline. Three series of injections were made in each of two animals; there was no evidence of potentiation of either hormone by the other.

The possible potentiation of the effects of angiotensin II and noradrenaline by tyramine was studied in eight experiments. Tyramine (2–4 mg) alone raised the blood pressure, on average, by 26 mm Hg and its effect was observable for up to 4 min. Angiotensin II (10 μg) and noradrenaline (20 μg) produced rises of 25 and 27 mm Hg respectively. When tyramine was combined with 10 μg of angiotensin II the average maximum pressor response was 49 mm Hg and when combined with 20 μg of noradrenaline the rise in blood pressure was 51 mm Hg.

Sequential injections of tyramine and the hormones showed that if tyramine preceded the injection of the angiotensin and noradrenaline by 4 min their pressor effects were enhanced (angiotensin II, on average, by 22 % and noradrenaline by 11 %).

Our findings indicate that mixtures of angiotensin II and noradrenaline are much more effective in raising the blood pressure of sheep than either hormone itself; however, this does not necessarily indicate that one potentiates the other; their mode of action on the small blood vessels differs (Peart, 1965) and this may explain the present results. Injections of mixtures of tyramine and angiotensin II, and of tyramine and noradrenaline, were also more effective than any of these compounds alone.

Sensitization to the pressor effects of angiotensin II by tyramine may be related to the noradrenaline-releasing action of tyramine and the observed potency of mixtures of angiotensin II and noradrenaline are in keeping with this suggestion.

These studies were made possible by the expert technical assistance of Mr. J. Wilson and Mr. O. F. Mason.

*Departments of Medicine and Metabolic Medicine,** E. C. OSBORN
Welsh National School of Medicine, G. TILDESLEY
Cardiff Royal Infirmary, L. P. O'GORMAN
Cardiff, U.K. R. F. MAHLER

February 13, 1971

REFERENCES

DAY, M. D. & OWEN, D. A. A. (1968). *Br. J. Pharmac.*, **34**, 499–507.
LOUIS, W. J. & DOYLE, A. E. (1966). *Clin. Sci.*, **31**, 247–254.
MCCUBBIN, J. W. & PAGE, I. H. (1963a). *Science, N.Y.*, **139**, 210–211.
MCCUBBIN, J. W. & PAGE, I. H. (1963b). *Circulation Res.*, **12**, 553–559.
OSBORN, E. C., HUGHES, S. G., PIRIE, A. T., WILLICOMBE, P. R., & MAHLER, R. F. (1969). *Cardiovasc. Res.*, **3**, 134–146.
PEART, W. S. (1965). *Pharmac. Rev.*, **17**, 143–182.
SCROOP, G. C. & WALSH, J. A. (1968). *Aust. J. exp. Biol. med. Sci.*, **46**, 573–580.

* Correspondence to Dr. E. C. Osborn at this address.

The effect of angiotensin I and II on hind-limb blood flow in sheep

It was shown by Ng & Vane (1967; 1968) that angiotensin I was substantially removed during a single passage through the hind-limb of the dog but without decreasing blood flow through the limb, and these authors suggested that it required conversion to angiotensin II in the pulmonary circulation before it acquired biological activity. However, earlier studies (Carlini, Picarelli & Prado, 1958; Halvorsen, Fasciolo & Calvo, 1959; Gross & Turrian, 1960; Barac, 1962) using angiotensin I of biological origin had shown that angiotensin I itself reduced the blood flow after perfusion of the hindlimb or hindquarters of the dog and of other species. In this study we report the effect of a synthetic angiotensin I, identical with human angiotensin I, on the hind-limb blood flow of the sheep.

The angiotensin was supplied by Schwarz BioResearch, Orangeburg, New York. It was aspartyl^{1}-isoleucyl^{5}-angiotensin I and was synthesized by the solid-phase technique pioneered by Merrifield (1963). Gel chromatography was used to purify the material and thin-layer chromatography and amino-acid analysis (Spackman, Stein & Moore, 1958) to establish its nature. These procedures indicated that less than 5% of impurities were present (Schwarz BioResearch, 1970; Dr. W. C. Roberts, personal communication). This angiotensin I preparation was tested for the presence of angiotensin II by comparing its action on the rat isolated colon with that of angiotensin II (asparaginyl^{1}-valyl^{5}-angiotensin II, Hypertensin, Ciba, Basle). This assay preparation responds weakly, or not at all, to angiotensin I (Osborn, Tildesley & others; unpublished observations). The results showed that the angiotensin I contained less than 1% of angiotensin II.

The animals we used were Kerry Hill and Welsh Mountain rams, wethers and ewes of average weight 28 kg (s.d. = $\pm$4 kg). Anaesthesia and monitoring of blood pressure were as described previously (Osborn, Hughes & others, 1969). Hind-limb blood flow was determined by a method involving direct collection of femoral vein blood.*

The effects of both hormones were studied in ten experiments. The animals were initially given several injections of saline and of 2 μg of angiotensin II into the femoral artery to accustom them to the procedure. When good reproducibility had been

* Full details on request.