Short communications

Effect of blood on the response of the rabbit ear artery to noradrenaline and angiotensin II

K. K. F. Ng, Y. F. Teh and R. F. Whelan*

Department of Pharmacology, University of Singapore, Singapore 3.

Blood or plasma added to the perfusing Krebs solution potentiated the vasoconstrictor effects of noradrenaline and angiotensin II on the isolated ear artery of the rabbit. The relatively greater increase in the vasoconstrictor effect of angiotensin II in the presence of blood or plasma may account for part of its potent pressor activity *in vivo*.

The vasoconstrictor effects of drugs on the isolated ear artery of the rabbit have so far been studied on preparations perfused with artificial physiological solutions. We report here the vasoconstrictor effects of noradrenaline and angiotensin II on this preparation when blood or plasma is added to the perfusing solution.

Methods.—Rabbits of either sex weighing 1·2–1·5 kg were anaesthetized with urethane (2 g/kg i.p.). Heparin (500 iu/kg) was injected into the marginal ear vein. Blood was collected from a cannula inserted into the lower abdomial aorta. In experiments where plasma was used, the blood was centrifuged at 3,000 r.p.m. for 15 minutes.

The central ear artery of the rabbit was isolated (de la Lande & Rand, 1965) and cannulated at both ends according to the method previously described (de la Lande, Cannell & Waterson, 1966). The artery was perfused with Krebs solution at a constant rate of 5–7 ml/minute. The Krebs solution was maintained at 37° C and equilibrated with 95% oxygen and 5% carbon dioxide.

When required, blood or plasma was infused into the perfusing Krebs solution. Noradrenaline bitartrate (Koch Light) and angiotensin II amide (Hypertensin, CIBA) were used. These were dissolved in 0·9% NaCl (w/v) and injected in volumes of 0·05–0·2 ml at 3–5 min intervals into the perfusing fluid. Vasoconstriction measured in terms of perfusion pressure (1 mmHg≡ 1·333 mbar) was recorded either by a mercury manometer on a smoked drum or by a Statham pressure transducer P23AC connected to a Grass polygraph.

Results.—The isolated central artery of the rabbit ear perfused with Krebs solution was highly sensitive to the vasoconstrictor action of noradrenaline. The threshold dose was 2·5–10·0 ng and the responses were dose dependent. By contrast, the threshold dose for angiotensin II was between 100–1000 ng. Four out of seven preparations became refractory to the effect of angiotensin II after the first or second dose (100–200 ng). In the remaining three preparations, however, a dose-response curve to angiotensin II (10–1000 ng) was obtained. In these preparations, noradrenaline on a weight basis was 40–100 times more potent than angiotensin II.

When the isolated ear arteries were perfused with whole blood or plasma, the blood vessels went into abrupt spasm making observations impossible. Experiments were therefore tried with blood or plasma added to the Krebs solution in the proportion of 1:10 (v/v). This perfusing medium produced a rise in the basal perfusion pressure (5–20 mmHg) and potentiated the vasoconstrictor effects of both noradrenaline and angiotensin II. The vasoconstrictor effect of noradrenaline was potentiated 4-fold and that of angiotensin II 8-fold. The results obtained with plasma (four experiments) were qualitatively similar to those obtained with blood (three experiments). The potentiating effect of blood or plasma was rapidly reversible; within 10–15 min of stopping the infusion, the potentiating effect of blood or plasma had disappeared.

Figure 1 shows the potentiating effect of blood on the vasoconstrictor responses produced by noradrenaline and angiotensin II. In Krebs solution containing blood (10:1), the effect of noradrenaline was increased 3–4 times and the effect of angiotensin II was increased more than 10 times. However, the ratio of potency of noradrenaline to angiotensin II decreased from approximately 1:150 in Krebs solu-

*Present address: Department of Human Physiology and Pharmacology, University of Adelaide, South Australia 5001.

tion to 1:20 in Krebs solution containing blood. Thus although the responses of both noradrenaline and angiotensin II were potentiated in the presence of blood the increase in response to angiotensin II was 8 times greater.

Discussion.—These results show that the isolated ear artery of the rabbit perfused with Krebs solution is far more sensitive to noradrenaline than to angiotensin II. The addition of blood or plasma to Krebs solution enhanced the vasoconstrictor effects of both of these drugs. The mechanism of potentiation is not clear at present. However, plasma proteins (Wurzel, Bacon, Kalt & Zweifach, 1964) sensitize isolated vascular strips to vasoconstrictor agents. 5-Hydroxytryptamine (0·5–10·0 ng/ml) has also been reported to potentiate the action of noradrenaline and angiotensin II on the isolated ear artery of the rabbit (de la Lande, *et al.*, 1966). Both the plasma proteins and the high concentration of 5-hydroxytryptamine in rabbit blood (3·5–9·4 μg/ml) and in rabbit plasma (0·9 μg/ml) (Garattini & Valzelli, 1965) may therefore contribute to the potentiating activity of blood or plasma.

The relatively greater increase in the vasoconstrictor effects of angiotensin II brought about by the addition of blood or plasma to Krebs solution may explain the difference in the effects of angiotensin II and noradrenaline on intact animals and isolated preparations. Thus although angiotensin II is only 1/150 as potent as noradrenaline on the isolated rabbit ear artery it is 10–20 times more potent on the blood pressure of laboratory animals (Gross, Khairallah, McGiff & Bunag, 1968). It is therefore probable that part of this greater potency is due to the potentiating effect of blood on the action of angiotensin II on vascular smooth muscles.

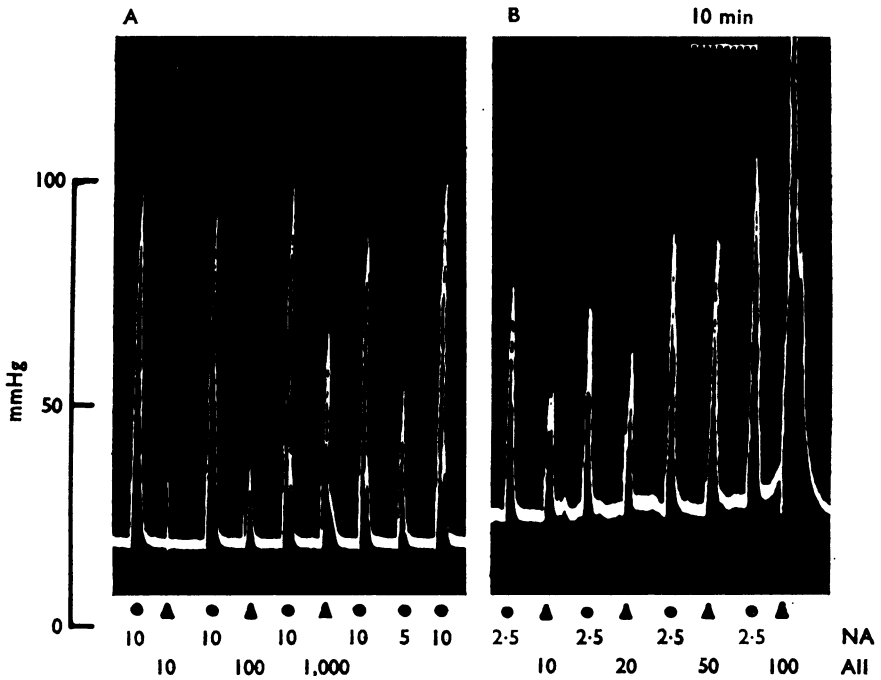

FIG. 1. Effect of blood on the vasoconstrictor responses of noradrenaline (NA) and angiotensin II (A II) on the isolated ear artery of the rabbit. All doses are in nanograms. Panel A shows responses obtained in Krebs solution. Panel B shows responses obtained 10 min after blood was added to Krebs solution. The ratio of potency of noradrenaline to angiotensin II in Krebs solution was approximately 1:150 (Panel A). The ratio was decreased to 1:20 (Panel B) in Krebs solution containing blood (10:1). Time: 10 min; vertical scale, mmHg.

REFERENCES

DE LA LANDE, I. S. & RAND, M. J. (1965). A simple isolated nerve-blood vessel preparation. *Aust. J. exp. Biol. med. Sci.*, **43**, 639–659.

DE LA LANDE, I. S., CANNELL, V. A. & WATERSON, J. G. (1966). The interaction of serotonin and noradrenaline on the perfused artery. *Br. J. Pharmac. Chemother.*, **28**, 255–272.

GARATTINI, S. & VALZELLI, L. (1965). In: *Serotonin*, pp. 262–263. Amsterdam: Elsevier Publishing Co.

GROSS, F., KHAIRALLAH, P. A., MCGIFF, J. C. & BUNAG, R. D. (1968). Pharmacology of angiotenson. In: *Renal Hypertension*, ed. Page, I. H. & McCubbin, J. W., pp. 134–159. Chicago: Year Book Medical Publishers, Inc.

WURZEL, M., BACON, R. C., KALT, R. B. & ZWEIFACH, B. W. (1964). Vasocative properties of plasma protein fractions. *Am. J. Physiol.*, **206**, 923–925.

(*Received April* 5, 1971)