Interaction of angiotensin II with catecholamines
in the circulation of the dog and cat

K. K. F. NG, S. DUFFY, W. J. LOUIS AND A. E. DOYLE
Department of Medicine, University of Melbourne, Austin Hospital, Heidelberg, Victoria, Australia

Summary
1. The blood-bathed organ technique was employed to study the effects of angiotensin II and catecholamines on an isolated everted rat aorta bathed in the extracorporeal circulating blood of adult dogs and cats.
2. When the injections were made into the bathing blood close to the everted rat aorta, angiotensin II was half as potent as adrenaline or noradrenaline on a molar basis.
3. After intravenous injections, the vasoconstrictor potency of angiotensin was twenty times that of adrenaline or noradrenaline on the everted rat aorta. The increase in potency was due to the interaction of angiotensin II with catecholamines on the preparation.
4. Intravenous phenoxybenzamine abolished the potentiated vasoconstrictor effect of angiotensin II on the blood-bathed everted rat aorta, but it did not abolish the pressor effect of angiotensin II on the cardiovascular system of the animals. The results suggest that catecholamines released into the circulating blood by intravenous angiotensin II do not play an important role in the pressor effect of angiotensin II.

Key words: angiotensin II, blood-bathed organ technique, catecholamines, circulation, everted rat aorta.

Introduction
Angiotensin II is the most potent vasopressor agent available. On a molar basis, it is forty to fifty times more potent than noradrenaline when given intravenously (De Bono, Lee, Motttram, Pickering, Brown, Keen, Peart & Sanderson, 1963). The vasopressor activity of angiotensin II is often attributed to its direct effect on the blood vessels and the indirect effect through the stimulation of the sympathoadrenal system. The separation of the direct and indirect effects of angiotensin II on the blood vessels in vivo has therefore met with difficulty in the past. We report the use of a new method which allowed us to distinguish between the direct effect of angiotensin II and the indirect effect mediated by blood-borne catecholamines on an isolated blood vessel bathed in extracorporeal circulating blood.

Methods
The blood-bathed organ technique (Vane, 1969) was used to assay angiotensin II and catecholamines continuously in the blood stream. Adult dogs and cats of either sex were anaesthetized with pentobarbitone. Heparinized blood was sampled from a femoral artery and pumped at a rate of 10 ml/min through an organ bath in which an everted rat aorta was mounted (Ng, Duffy, Louis & Doyle, 1975). The blood overflowed from the top of the bath to a venous reservoir, returning to the vein of the animal by gravity feed. In some experiments, the blood was allowed to superfuse (Gaddum, 1953) in series a chick rectum (Mann & West, 1950) and a rat colon (Regoli & Vane, 1966) for the assays of adrenaline and angiotensin II respectively (Vane, 1969).

The everted rat aorta was prepared and set up as previously described (Ng et al., 1975). One end of the blood vessel was occluded while the other end was sleeved over a cannula which in turn was connected to a pressure transducer via a three-way tap. The lumen of the blood vessel was filled with Krebs solution and the increase in intraluminal pressure was
amplified and recorded on a polygraph. The systemic blood pressure and the movements of the other two assay tissues were continuously monitored.

Two groups of experiments were carried out with this technique. In the first group of experiments, the direct vasoconstrictor potency of angiotensin II was compared with that of catecholamines on the everted rat aorta. This was done by injecting into the bathing blood close to the everted rat aorta. In the second group of experiments, the comparison of the vasoconstrictor potency of angiotensin II with catecholamines on the everted rat aorta was made after the injections were given into the vein of the animals. In this way, the direct effect of angiotensin II on the everted rat aorta and the indirect effect mediated by other blood-borne vasoconstrictors could be estimated.

Results

The everted rat aorta was selectively sensitive to angiotensin II, adrenaline and noradrenaline. Substances such as acetylcholine, histamine, serotonin, bradykinin and prostaglandins E₁, E₂ and F₂, were relatively inactive on the preparation. Injections of angiotensin II, adrenaline and noradrenaline (0.01–0.1 nmol) into the bathing blood induced dose-dependent responses on the everted rat aorta. These injections had no effect on the systemic blood pressure of the animals. Adrenaline, however, could be distinguished by its relaxant effect on the chick rectum and angiotensin II could be distinguished by its constrictor effect on the rat colon. The comparisons made by injections into the bathing blood showed that angiotensin II was half as potent as adrenaline; the equimolar potent dose ratio of adrenaline to angiotensin II was 0.5:1 (mean value of eight experiments). However, when equimolar doses of angiotensin II and adrenaline (0.2–20.0 nmol) were injected intravenously, the effects of angiotensin II on the everted rat aorta were twenty times greater than those produced by adrenaline or noradrenaline; the equipotent molar dose ratio of adrenaline to angiotensin II was 10:1 (mean value of eight experiments). The results of one such experiment are shown in Fig. 1.

What causes the increased vasoconstrictor potency of angiotensin II after intravenous injections? The everted rat aorta was bathed in femoral arterial blood so that angiotensin II injected intravenously had to pass through the lungs before reaching the assay organ. Since both angiotensin II and adrenaline traverse the lungs without any loss (Ng & Vane, 1968), the results suggested that angiotensin II released vasoconstrictors from the pulmonary circulation. The effects of intravenous injections of angiotensin II (0.2–2.0 nmol) on the everted rat aorta bathed in femoral arterial blood was therefore compared with those induced by injections given into root of the ascending aorta. Similar effects were produced on the everted rat aorta by equimolar doses of angiotensin II given intravenously or intra-aortically.
Thus angiotensin II did not release vasoconstrictors from the pulmonary circulation.

Angiotensin II, on the other hand, is a potent mediator for the release of catecholamines from the sympatho-adrenal system (Feldberg & Lewis, 1964). The increase in potency of angiotensin II after intravenous injections could be due to the release of catecholamines into the circulation, followed by its interaction with angiotensin II on the everted rat aorta. This was confirmed by subsequent experiments in which a chick rectum was included in the system for the bioassay of adrenaline: the vasoconstrictor effect produced by intravenous angiotensin II on the everted rat aorta was always associated with the relaxation of the chick rectum; this was evidence that adrenaline was released into the circulating blood. To what extent do blood-borne catecholamines contribute towards the systemic pressor response of angiotensin II?

The effects of intravenous angiotensin II and catecholamines on the blood-bathed everted rat aorta and on the systemic blood pressure were examined. In two cats, angiotensin II had negligible effects on the everted rat aorta but given intravenously it was twenty to forty times more potent than noradrenaline; the same order of potency was obtained on the blood pressure of the animals. Intravenous phenoxybenzamine hydrochloride (1 mg/kg) abolished the vasoconstrictor effects of intravenous angiotensin II on the everted rat aorta but the pressor effects of angiotensin II on the blood pressure of the animals remained unchanged. In contrast, the vasoconstrictor effects of noradrenaline on the everted rat aorta and on the systemic blood pressure of the animals were both abolished. The direct effect of angiotensin II on the everted rat aorta, however, was marginally reduced. Thus phenoxybenzamine blocked the effects of catecholamines on the everted rat aorta but it did not abolish the effects of angiotensin II on the preparation and on the systemic blood pressure.

Discussion

With the everted rat aorta bathed in circulating blood of the animals, the direct effect of angiotensin II and the indirect effect mediated by blood-borne catecholamines can be studied separately. These experiments show that, on a molar basis, angiotensin II is only half as potent as adrenaline or noradrenaline on the everted rat aorta. The relatively low potency of angiotensin II on the preparation is similar to the results previously obtained on the isolated rabbit ear preparation (Ng, Teh & Whelan, 1971). The results are also in agreement with those obtained in man, in whom intra-arterial infusion of angiotensin II was only half as potent as noradrenaline (Scroop, Walsh & Whelan, 1965).

The increase in vasoconstrictor potency of angiotensin II on blood vessels when administered by the intravenous rather than the intra-arterial route was first demonstrated in man (Scroop et al., 1965). However, previous results did not throw light on whether the increase in potency was due to the action of angiotensin II alone or whether it was due to its interaction with catecholamines released from the sympatho-adrenal system. We have now demonstrated that this increase in potency on the isolated blood vessel bathed in extracorporeal blood is due to the interaction of angiotensin II with circulating catecholamines. We have also demonstrated that, after a-adrenoreceptor blockade in the circulation of the animals and on the blood-bathed everted rat aorta, the blood-borne catecholamines released by angiotensin II do not play an important role in the angiotensin II pressor response. The results therefore suggest that angiotensin II acts on receptors in the blood vessels in vivo such that its vasoconstrictor effect over-rides those produced by catecholamines released from the sympatho-adrenal system.

Acknowledgments

This work was supported by grants from the National Heart Foundation of Australia. K.K.F.N. held the Warren McDonald International Fellowship of the National Heart Foundation of Australia.

References


Ng, K.K.F., Duffy, S., Louis, W.J. & Doyle, A.E. (1975) Everted aorta: a new method for the bioassay of vaso-