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February 13, 1971

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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 This assay preparation responds weakly, or not at all, to angiotensin I (Osborn, Tildesley & others; unpublished observations).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.

achieved (i.e. values usually within $\pm 10\%$ of the means), angiotensin I (12 μg) was compared with various doses (0·5, 1·0, 2·0, 5·0 and 10 μg) of angiotensin II.

The procedure was as follows: each dose of angiotensin was alternated with one of saline at least 5 min after the injection of the hormone; longer periods were allowed for the larger doses. Two injections of angiotensin I were made initially. These were followed by two injections of each of any three of the five doses of angiotensin II. These were followed by a further two injections of angiotensin I after which the remaining two doses of angiotensin II were employed, each dose being injected twice. A further two injections of angiotensin I were than made to conclude the experiment.

The hind-limb blood flow in the control periods averaged 1·1 ml/s (s.d. = $\pm 0{\cdot}2$ ml/s). Both angiotensin I and angiotensin II caused an immediate fall in hind-limb blood flow; the effects of the injections of the hormones are shown in Table 1. The percentage reduction in blood flow has been calculated relative to the flow during the control period preceding and after the injection of each hormone. On average, angiotensin I (12 μg) had the same effect as a dose of 1–2 μg of angiotensin II.

The replication of the procedure was tested with 1·0 and 0·3 μg of angiotensin II in ten other animals of about the same size. These doses were chosen to give reductions in blood flow about equal to, and appreciably less, than those given by 12 μg of angiotensin I. Injections were made in the sequence saline, 0·3 μg of angiotensin II, saline and 1·0 μg of angiotensin II. Ten series of injections were made in each experiment and the effect of the angiotensin injection was calculated with respect to the two control values before and after it. The results (Table 2) were analysed in terms of five pairs of injections for each dose. The average control blood flow in these studies was 1·0 ml/s (s.d. = $\pm 0{\cdot}3$ ml/s). The results indicate that the method has acceptable reproducibility over the range of blood flow reductions reported in Table 1.

Table 1. *The reduction in hind-limb blood flow in ten sheep after injections of angiotensin I and angiotensin II into the femoral artery.* The s.d. is given in brackets.

Hormone	Dose (μg)	Reduction in blood flow (%)
Angiotensin I	12	24 (± 7)
	0·5	19 (± 5)
	1·0	23 (± 5)
Angiotensin II	2·0	27 (± 6)
	5·0	30 (± 5)
	10	30 (± 6)

Table 2. *The reductions in hind-limb blood flow after injections of* 0·3 *and* 1·0 μg *of angiotensin II into the femoral artery in ten sheep.* The s.d., for the five paired estimations with each dose, is given in brackets. The experiments have been arranged from left to right order of increasing sensitivity to the smaller dose of the hormone. Analysis of variance (Fisher & Yates, 1948; Moroney, 1951) showed that the method could distinguish ($P < 0{\cdot}05$ or $\simeq 0{\cdot}05$) the effects of the two doses in seven of the experiments.

Dose of angiotensin II (μg)	Reduction in blood flow (%)										Mean and s.d.
0·3	8 (± 2)	12 (± 3)	13 (± 3)	14 (± 3)	15 (± 4)	18 (± 4)	18 (± 4)	18 (± 3)	26 (± 4)	31 (± 6)	17 (± 7)
1·0	11 (± 2)	19 (± 4)	17 (± 3)	22 (± 3)	21 (± 3)	21 (± 2)	29 (± 2)	31 (± 2)	31 (± 4)	38 (± 5)	24 (± 8)

The present investigations show that the injection of either hormone into the hind-limb has an immediate effect on blood flow but that angiotensin I is much less effective than angiotensin II. We have found in other investigations that both hormones used in the present studies are removed equally by the hind-limb of the sheep. A possible explanation for the present findings is that much of the angiotensin I is inhibited or destroyed rather than converted to angiotensin II when it is removed from the circulation. Our findings in the sheep differ from those of Ng & Vane (1968) in the dog and they are in agreement with those reported in several species by the earlier workers. These studies were made in the Dr. Leonard West Research Laboratory of Sully Hospital, Sully, Glamorgan, and we gratefully acknowledge the expert technical assistance of Mr. J. Wilson, Mr. O. F. Mason and Mr. P. Stock.

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Is 5-hydroxytryptamine involved in the mechanism of action of fenfluramine?

Jespersen & Scheel-Krüger (1970) recently reported that methysergide blocked the hypothermic effect of fenfluramine in dogs and concluded that 5-hydroxytryptamine (5-HT) played an important role in the mechanism of action of fenfluramine, an anorectic drug that does not produce central stimulation in most animals (Le Douarec, Schmitt & Laubie, 1966). On the other hand, Opitz (1967) found that fenfluramine inhibited the appetite of rats in which brain 5-HT had been depleted by *p*-chlorophenylalanine, an experiment that strongly suggested that 5-HT was not required for the action of fenfluramine.