

Effect of rabbit antibodies against angiotensin-II on the pressor response to angiotensin-II and renal hypertension in the rat

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1. In the intact or nephrectomized rat, the pressor responses to synthetic angiotensin-II-amide, beta-angiotensin-II and native "rat angiotensin" could be inhibited by intravenous injection of rabbit serum containing antibodies against synthetic angiotensin-II-amide.
 2. The pressor responses to vasopressin or noradrenaline were not influenced by anti-angiotensin serum.
 3. A single injection of anti-angiotensin serum inhibited the pressor responses to angiotensin-II-amide for a period of 2.5 hr.
 4. Intravenous infusion or daily intraperitoneal injection of anti-angiotensin serum did not influence blood pressure levels in renal hypertensive rats.
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Increased renin production and release and angiotensin formation have been postulated as a contributory factor in the development or maintenance of hypertension. In certain forms of experimental renal hypertension, increased renin activity has been demonstrated in the kidneys and in circulating blood (Goldblatt, 1947). Conversely, prolonged administration of suitable doses of synthetic angiotensin-II in the rabbit or rat can produce a steadily increasing or sustained rise in blood pressure over a period of days or months (Brown, Chapius & Robertson, 1963; Dickinson & Lawrence, 1963; Marx, Deane, Mowles & Sheppard, 1963).

Circulating antibodies against angiotensin-II can be induced in the rabbit by immunization with conjugates of angiotensin-II with protein or synthetic polypeptides (Deodhar, 1960; Goodfriend, Levine & Fasman, 1964; Haber, Page & Jacoby, 1965), and the immunological properties of these antibodies have been determined *in vitro* (Goodfriend *et al.*, 1964; Dietrich, 1967).

In the present investigation, the influence of rabbit anti-angiotensin serum on the pressor response to angiotensin-II was determined, and the effect of massive doses of rabbit anti-angiotensin serum on the blood pressure of renal hypertensive rats was studied.

Methods

Sera

Rabbit anti-angiotensin sera were prepared by immunization with angiotensin-II-protein conjugates in complete Freund's adjuvant, followed by several intravenous

injections of alum-precipitated angiotensin-II. Antisera from six rabbits were pooled, and portions of 2 ml. were stored at -20° C until use. *In vitro* anti-angiotensin activity has been demonstrated previously using a modified ammonium sulphate precipitation technique (Dietrich, 1967). Sera from normal, untreated rabbits for use in control experiments were pooled and stored in the same manner.

Effect on the pressor response to angiotensin

The pressor response to synthetic angiotensin-II-amide (angiotensin-II) and beta-angiotensin-II (Brunner & Regoli, 1962) and to "rat angiotensin" were determined in bilaterally nephrectomized or intact, normotensive rats. "Rat angiotensin" was obtained by incubation of plasma from nephrectomized rats with hog renin in the presence of EDTA at pH 5.5.

Bilateral nephrectomy was carried out under ether anaesthesia 16–20 hr before some experiments. Blood pressure in a carotid artery was measured under urethane anaesthesia (1.7 g/kg subcutaneously) with a mercury manometer. Test substances were injected into a jugular vein in a constant volume of 0.4 ml./rat. The maximal increase in blood pressure produced by each pressor substance before and 15 min after injection of anti-angiotensin serum or control serum was compared in the same animal.

The influence of antiserum or control serum on the pressor responses to synthetic vasopressin and noradrenaline was studied in the same manner.

The duration of angiotensin-II inhibition was determined in the intact, normotensive rat under urethane anaesthesia. Three test doses of angiotensin-II were injected. After injection of anti-angiotensin serum or control serum, the injection of the three test doses of angiotensin was repeated at 30 min intervals for a period of 2.5 hr.

Effect on blood pressure in the renal hypertensive rat

Renal hypertension was produced by constriction of one renal artery under ether anaesthesia with a silver clip. The animals were used for the experiments at least four weeks after the operation, when stable near-systolic blood pressure levels above 189 mm Hg had been reached.

The effect of intravenous injection and subsequent infusion of rabbit anti-angiotensin serum or normal rabbit serum was studied in the unanaesthetized hypertensive rat. Animals were used 10 to 12 weeks after constriction of the renal artery. Under pentobarbital anaesthesia (Nembutal, Abbott 50 mg/kg intraperitoneally) a polyethylene cannula was implanted into the abdominal aorta using the method of Weeks & Jones (1960). Mean blood pressure in the lightly restrained animals was directly measured 5 to 7 days later with a pressure transducer. A single intravenous injection of anti-angiotensin serum was given, followed by infusion of anti-angiotensin serum for 2 hr. Control animals received the same dose of normal rabbit serum. Sera were injected and infused into a tail vein.

In a second group of renal hypertensive rats, near-systolic blood pressure was measured under light ether anaesthesia by the plethysmographic method of Byrom & Wilson (1938). Rats were used 4 weeks after constriction of the renal artery. During a period of 4 days the rats received daily interperitoneal injections of

anti-angiotensin serum or normal rabbit serum. Blood pressure was measured before and 2 hr after each daily injection.

Drugs

Synthetic asp¹-(NH₂)-val⁵ angiotensin-II (Hypertensin, CIBA) and lys⁸ vasopressin (Sandoz), hog renin (Nutritional Biochemicals Corp.) and (-)-noradrenaline hydrochloride (Arterenol, Hoechst) were used.

Results

Inhibition of the pressor response to angiotensin

The pressor effects of the various test substances before serum injection are given in Table 1. The pressor responses to each test substance did not differ significantly in the two groups.

TABLE 1. Control pressor responses in the nephrectomized rat before treatment with sera

Test substance and dose	Pressor response (mm Hg)	
	Control group	Treatment group
Angiotensin-II		
0.04 $\mu\text{g}/\text{kg}$ i.v.	32 \pm 5 (8)	30 \pm 2 (18)
0.16 $\mu\text{g}/\text{kg}$ i.v.	50 \pm 5 (8)	49 \pm 3 (18)
Beta-angiotensin-II		
0.04 $\mu\text{g}/\text{kg}$ i.v.	48 \pm 4 (6)	57 \pm 4 (7)
"Rat angiotensin"	52 \pm 5 (6)	46 \pm 5 (9)
Vasopressin		
0.03 u./kg i.v.	47 \pm 9 (8)	38 \pm 5 (18)
Noradrenaline		
6 $\mu\text{g}/\text{kg}$ i.v.	46 \pm 3 (6)	36 \pm 5 (9)

Values given are mean \pm S.E.M. (n).

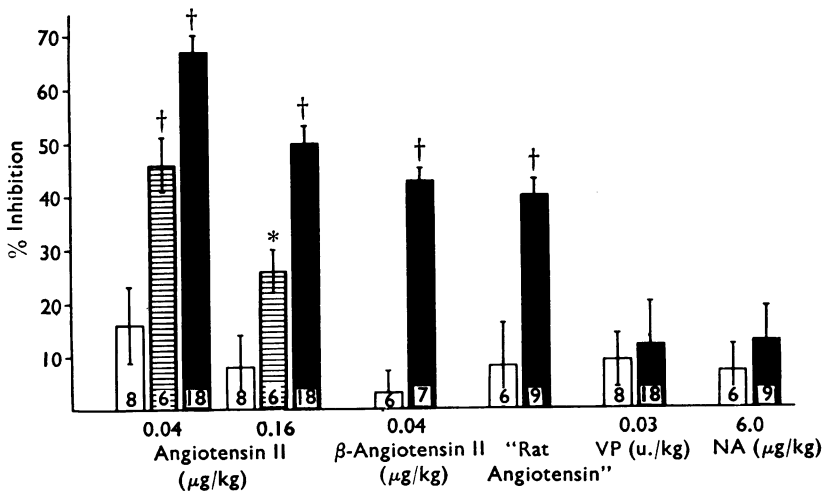


FIG. 1. Influence of anti-angiotensin serum or control serum on the pressor responses to angiotensin-II, beta-angiotensin-II, "rat-angiotensin," vasopressin (VP) and noradrenaline (NA) in the nephrectomized rat. The columns represent the mean inhibition of the pressor response in % of the control response; the cross bars represent S.E.M. Open columns, control serum 1 ml./kg i.v.; shaded columns, anti-angiotensin serum 0.5 ml./kg; black columns, anti-angiotensin serum 1 ml./kg i.v. The numbers in the lower portion of the columns indicate the number of animals in each group. *, Significant at $P < 0.01$; †, significant at $P < 0.001$.

In the nephrectomized rat, anti-angiotensin serum 0.5 ml./kg inhibited the response to angiotensin-II 0.04 $\mu\text{g}/\text{kg}$ by 46% (Fig. 1). With the higher dose of 1 ml./kg, a more pronounced inhibition of the pressor response to this dose of angiotensin-II was obtained. The effect of angiotensin-II 0.16 $\mu\text{g}/\text{kg}$ was decreased to a lesser extent than that of the lower dose of angiotensin-II. The pressor response to beta-angiotensin-II 0.04 $\mu\text{g}/\text{kg}$, which was more pronounced than that to angiotensin-II 0.04 $\mu\text{g}/\text{kg}$, was inhibited to a smaller extent. The degree of inhibition of beta-angiotensin-II corresponded to that of an equipressor dose (0.16 $\mu\text{g}/\text{kg}$) of angiotensin-II. The response to "rat angiotensin" was inhibited to roughly the same extent as an equipressor dose of angiotensin-II. Anti-angiotensin serum did not significantly influence the pressor effects of vasopressin or noradrenaline ($P > 0.05$). Normal rabbit serum did not affect the pressor response to any of the test substances ($P > 0.05$).

In the intact, non-nephrectomized rat anti-angiotensin serum 1 ml./kg inhibited the effects of angiotensin-II 0.04 and 0.16 $\mu\text{g}/\text{kg}$ to the same extent, while normal rabbit serum had no effect (Table 2).

In a group of intact rats, the influence of a single injection of anti-angiotensin 1 ml./kg on the responses to three test doses of angiotensin-II was determined at 30 min intervals. The pressor response to angiotensin-II 0.05 $\mu\text{g}/\text{kg}$ was inhibited by 54% 30 min after injection of anti-angiotensin serum, and this degree of inhibition remained fairly constant for a period of 2.5 hr (Table 3). Inhibition of the

TABLE 2. Influence of anti-angiotensin serum and control serum on the pressor response to angiotensin-II in the intact rat

Treatment	Control pressor response (mm Hg) Angiotensin-II $\mu\text{g}/\text{kg}$ i.v.		% inhibition of the pressor response 15 min after injection of serum Angiotensin-II $\mu\text{g}/\text{kg}$ i.v.	
	0.04	0.16	0.04	0.16
Normal rabbit serum 1.0 ml./kg i.v. (6)	14 \pm 1	27 \pm 3	7 \pm 23	9 \pm 10
Anti-angiotensin serum 1.0 ml./kg i.v. (6)	20 \pm 1	30 \pm 2	43 \pm 6*	47 \pm 7*

Values given are mean \pm S.E.M. (n). * Significant at $P < 0.001$.

TABLE 3. Duration of the influence of a single injection of anti-angiotensin serum or control serum on the pressor response to angiotensin-II in the intact rat

Treatment	Angiotensin-II test dose $\mu\text{g}/\text{kg}$ i.v.	Change in pressor response (in % of control response) after injection of serum				
		30 min	60 min	90 min	120 min	150 min
Normal rabbit serum 1.0 ml./kg i.v. (6)	0.04	-20 \pm 17	-27 \pm 17	-20 \pm 22	-17 \pm 24	+3 \pm 34
	0.16	-8 \pm 4	-3 \pm 10	+13 \pm 7	+14 \pm 9	+26 \pm 14
	0.64	+7 \pm 8	+10 \pm 11	+21 \pm 6†	+29 \pm 7*	+36 \pm 7†
Anti-angiotensin serum 1.0 ml./kg i.v. (6)	0.04	-54 \pm 6‡	-35 \pm 15*	-38 \pm 9†	-46 \pm 6‡	-53 \pm 11†
	0.16	-42 \pm 4‡	-40 \pm 8†	-26 \pm 11	-26 \pm 6†	-33 \pm 8†
	0.64	-12 \pm 10	-17 \pm 16	-2 \pm 13	-18 \pm 12	+7 \pm 10

Values given are mean \pm S.E.M. (n). * Significant at $P < 0.05$. † $P < 0.01$. ‡ $P < 0.001$.

intermediate test dose of angiotensin-II was less pronounced, but significant inhibition could still be demonstrated after 150 min. The effect of the highest angiotensin-II dose was not influenced. In a parallel group of control rats treated with normal rabbit serum, the pressor responses to angiotensin-II increased steadily during a period of 2.5 hr.

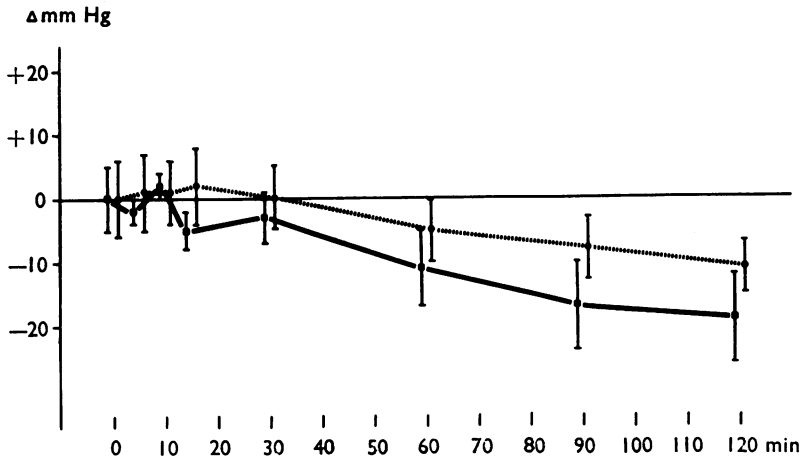


FIG. 2. Influence of intravenous injection of anti-angiotensin serum 2 ml./kg and infusion of 1 ml./kg per hr for 2 hr (solid line) and of equivalent amounts of control serum (dotted line) on the blood pressure of the unanaesthetized renal hypertensive rat. The points represent the mean change in blood pressure in mm Hg; the cross bars represent S.E.M. The mean initial blood pressure in the control group was 169 ± 6 mm Hg and 170 ± 5 mm Hg in the treatment group. Each group consisted of nine animals.

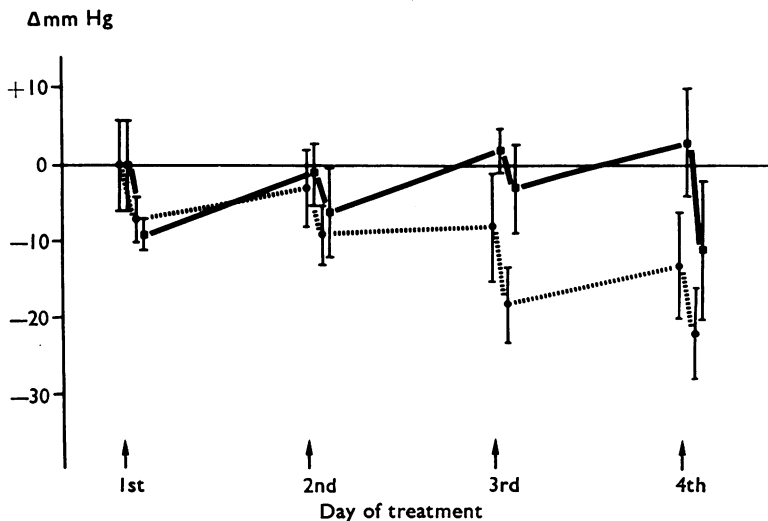


FIG. 3. Influence of daily intraperitoneal injection of anti-angiotensin 5 ml./kg (solid line) or control serum 5 ml./kg (dotted line) on the blood pressure of the renal hypertensive rat. Blood pressure was measured before and 2 hr after each daily injection (arrows). The initial near-systolic blood pressure in the control group was 204 ± 6 mm Hg and 205 ± 6 mm Hg in the treatment group. Each group consisted of six animals. All other designations as in Fig. 2.

Effect in normotensive rats

In normotensive, nephrectomized or intact rats, injection of anti-angiotensin serum produced occasional decreases in blood pressure lasting less than 15 min. Similar effects were seen after injection of normal rabbit serum.

Effect in renal hypertensive rats

Intravenous injection of anti-angiotensin serum 2 ml./kg and subsequent infusion of 1 ml./kg per hr for 2 hr had no antihypertensive effect in the unanaesthetized, renal hypertensive rat (Fig. 2). The slight decrease in blood pressure of 19 mm Hg seen after 2 hr corresponded to the fall in blood pressure in control animals treated with the same dose of normal rabbit serum. Similarly no significant change in the blood pressure values measured in light ether anaesthesia was found in renal hypertensive rats treated daily for 4 days with anti-angiotensin serum 5 ml./kg intraperitoneally (Fig. 3). In control animals treated with normal rabbit serum, a more pronounced fall in blood pressure was seen.

Discussion

The pressor response to angiotensin-II in the nephrectomized rat could be inhibited by injection of serum from rabbits immunized against angiotensin-II. This effect was roughly dose dependent, and similar inhibition of the effects of approximately equi-pressor doses of angiotensin-II, beta-angiotensin-II and "rat angiotensin" was found. The effect of anti-angiotensin serum was specific for angiotensin-II and its analogues, as the pressor effects of vasopressin and noradrenaline were not influenced.

In the intact rat the response to angiotensin-II 0.16 $\mu\text{g}/\text{kg}$ was inhibited by anti-angiotensin serum 1 ml./kg to the same extent as in the nephrectomized animal, while inhibition of a lower dose of angiotensin-II was less pronounced. In view of the relatively slight pressor effect of the lower dose of angiotensin-II in the non-nephrectomized rat, however, the exact degree of inhibition of this dose cannot be determined by this method. Relatively constant and long-lasting inhibition of angiotensin-II was produced by a single injection of anti-angiotensin serum, in spite of repeated injections of exogenous angiotensin-II and the ability of the intact rats to produce endogenous angiotensin. The dose-dependency of the inhibition of angiotensin-II therefore seems to be related to the relative amounts of angiotensin-II and antibodies and to the rapid administration of angiotensin-II rather than to depletion of antibodies.

Injection of anti-angiotensin serum produced no consistent effect on the blood pressure of the normotensive, nephrectomized or intact rat. The occasional, short-lasting decreases in blood pressure were also seen after injection of normal rabbit serum and may be attributable to an unspecific effect.

In studies with anti-renin, passive transfer of dog anti-renin serum produced an antihypertensive effect in the renal hypertensive dog (Wakerlin, 1958). In the rat, dog anti-renin serum inhibited the pressor response to renin, but did not reduce the blood pressure of renal hypertensive rats (Weiser & Hoobler, 1964).

Similarly, although rabbit anti-angiotensin serum was active against "rat angiotensin" in the rat, no effect on experimental renal hypertension was found. The

total intravenous dose of anti-angiotensin serum given in the unanaesthetized renal hypertensive rat was four times higher than that required to produce long-lasting inhibition of angiotensin-II in the normotensive rat and would seem sufficient to reveal, if only briefly, a possible role of endogenous angiotensin in the maintenance of elevated blood pressure.

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