An investigation of the prolonged pressor response to renin in the nephrectomized rat

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Summary

1. In an investigation of the prolonged pressor response to renin that develops after nephrectomy, angiotensin I dose-response curves and rat renin clearances were studied in nephrectomized rats and paired sham-nephrectomized control animals under pentobarbitone anaesthesia.

2. Both threshold and slope of the angiotensin I dose-response curves were decreased at 15–27 h after nephrectomy.

3. The ratio of renin clearance (determined during renin infusions) in the nephrectomized rat to that in the paired sham-nephrectomized control animals was 0.49 ± 0.03 (SEM), P < 0.001 (n = 12 pairs).

4. Both factors contribute towards the prolongation of the blood pressure increase after intravenously administered renin in the nephrectomized animal.

Key words: angiotensin I immunoassay, renin clearance, renin substrate.

Introduction

The well-recognized prolongation of the pressor response to intravenously administered renin, which develops in the rat over 24 h after nephrectomy, has never been satisfactorily explained. Studies on renin clearance are conflicting (Schaechtelin, Regoli & Gross, 1964; Peters-Haefeli, 1971), and even where a decreased clearance has been found its degree seems insufficient to account for the whole effect. It was therefore decided to re-investigate this phenomenon, particularly in the light of the observation that protein-binding of renin may greatly prolong its blood pressure response in vivo (Boyd, 1974).

Methods

Animals and materials

White male Wistar rats weighing 300–500 g were used in the study. They were anaesthetized with sodium pentobarbitone (Nembutal) given intraperitoneally in a dose of 6 mg/100 g. A neck incision was then made and the trachea cannulated for the administration of oxygen (approx. 0.3 ml/s). Both external jugular veins and the right common carotid artery were cannulated with polyethylene PE60 (Clay–Adams) cannulae and the basal blood pressure was lowered to approximately 50 mmHg by the administration of 1 mg of pentolinium tartrate given subcutaneously. The rats were given 100–150 units of heparin intravenously. Blood pressure was measured with a Statham strain gauge coupled to a Beckman Dynograph recorder (type RP). All blood samples for plasma renin concentration measurement were taken from the carotid artery cannula.

Bilateral nephrectomy (or sham nephrectomy) was carried out retroperitoneally through loin incisions under ether anaesthesia 15–27 h before the experiment proper. Water and food were withheld overnight but water was offered for 1–2 h on the morning of the experiment.

Rat renin was prepared either by the method of Peart, Lloyd, Thatcher, Lever, Payne & Stone (1966), up to and including the DEAE-cellulose step, or by trichloroacetic acid precipitation of rat kidney homogenates at pH 2.9 (room temperature). The final solution for infusion was made up in Tris buffer (0.02 mol/l), NaCl (0.13 mol/l), neomycin sulphate (0.3 mmol/l; 0.02%) and human serum.
albumin (0.05 mmol/l), pH 7.5. During experiments this renin was infused via a Braun Perfusor (model 871-104) at a rate of 0.05 ml/min in amounts sufficient to raise the blood pressure in the sham-nephrectomized animal by 40-60 mmHg.

Angiotensin I was the isoleucine5 form (Schwarz-Mann). 125I-labelled angiotensin I was obtained from the New England Nuclear Corp.

**Plasma renin concentration assay**

Rat renin substrate was prepared from 24-48 h nephrectomized rat plasma by dialysis against sodium phosphate buffer (0.025 mol/l), pH 7.5 (containing neomycin, 0.3 mmol/l), application to a 2 cm x 40 cm DEAE-cellulose column equilibrated in the same buffer, and elution with a buffer containing sodium phosphate (0.025 mol/l), sodium chloride (0.05 mol/l) and neomycin (0.3 mmol/l), pH 7.5. After pressure dialysis (Amicon PM 10 membranes) the substrate was dialysed against sodium phosphate buffer (0.1 mol/l) containing sodium chloride (0.05 mol/l), neomycin (0.3 mmol/l), pH 7.5. The final substrate generated between 3.5 and 6.6 μg of angiotensin I/ml when excess of renin was added. Its protein concentration was 20-25 mg/ml. If assay subsequently showed any appreciable 'angiotensinase' activity, greater than 20% loss of added (50 ng/ml) angiotensin I over 4 h at 37°C, the substrate was acidified (with 1 mol/l HCl) to pH 4.5 for 30 min at 32°C and then neutralized to pH 7.5 with NaOH (1 mol/l) (Skinner, 1967). For renin concentration assay rat plasma samples (0.025 ml) were incubated with 0.15 ml of substrate, dimercaprol (BAL) (0.01 mol/l), EDTA (0.015 mol/l) and phenylmethylsulphonyl fluoride (0.01 mol/l), in a final volume of 0.2 ml. After incubation for 4 h at 37°C renin was determined directly on unextracted plasma by immunoassay of the angiotensin I generated (Boyd, Adamson, Fitz & Peart, 1969).

**Results**

First, the slow development of a prolonged pressor response to intravenous renin over the 24 h post-nephrectomy period was confirmed. This effect was not present during the first hour, but appeared to develop to its maximum some 15-24 h after nephrectomy. An injection of renin would then increase blood pressure 40-100 mmHg with a slow decline towards base line over the next 30-60 min, whereas in the sham-nephrectomized animal a dose of approximately twice this amount of renin resulted in a slightly lesser peak blood pressure response with a decline to basal within 10-15 min.

**Dose-response curves to infusions of angiotensin I**

Angiotensin I was infused in nephrectomized and sham-nephrectomized rats at five different doses over the range 2.5-50 pmol/min, the infusions lasting approximately 10 min at each dose. The results showed a much lower threshold of effect and a less steep dose–response slope in the nephrectomized animals than in sham-nephrectomized control group, both of these factors favouring a prolongation of blood pressure response in the former for any given renin clearance rate. However, calculations showed that this factor alone was insufficient to account for the large discrepancy in blood pressure decline after intravenous injection of renin in these two situations.

**Plasma renin disappearance after intravenous injections of rat renin**

This was delayed to a variable extent in the nephrectomized animal, but because of the uncertain (and probably variable) effect of the decaying blood pressure on endogenous renin after injection, there was some doubt about the plasma renin concentrations due to the renin injection, particularly during the later phases of the curve. This made interpretation difficult, and further clearance studies were therefore done with renin infusions, where the continued angiotensin-mediated blood pressure elevation results in almost complete suppression of endogenous plasma renin.

**Plasma renin clearance calculated from renin infusions**

Rat renin was infused over 30–50 min, with two blood samples being taken 10 min apart over the last 15 min of infusion. It was confirmed that renin concentration had reached plateau within this time and so these two values were averaged for the calculation of renin clearance. Experiments were done and assayed in pairs and the results expressed as the ratio of renin clearance in the nephrectomized rat to that in its paired sham-nephrectomized control rat. The results showed that the renin clearance in the 15–27 h nephrectomized rat was reduced to approximately half that in the sham-
nephrectomized control rats at a mean ratio of 0.49 ± 0.003, \( P < 0.001 \) (\( n = 12 \) pairs).

This effect was independent of the dose of renin and therefore of the blood pressure response. Similar results were obtained independently from an indirect biological assessment of 'effective renin clearance'. This was calculated from pressor response to injected renin and the blood pressure dose-response curves to infused angiotensin in the same rat.

Discussion

At least two factors appear to be concerned in the greatly prolonged blood pressure response to renin that develops during the 24 h after nephrectomy.

(1) A decrease in threshold and in slope of the dose-response curve to angiotensin I, both factors tending to slow the fall of blood pressure following any given peak.

(2) A reduction in clearance of renin in the nephrectomized animal to approximately half of the value in sham-nephrectomized control rats. Experiments are under way to determine how much this reduction in renin clearance is due to the removal of the kidney per se, since the slow development of the prolonged blood pressure response could result from an immediate reduction in renin clearance, together with a slow onset of the change in the angiotensin response curve.

No evidence has been obtained so far to suggest that this prolongation of the blood pressure response to renin after nephrectomy is associated with renin binding to a protein that can be dissociated by relatively brief acidification (Boyd, 1974).

References


