SHORT COMMUNICATION

Effect of antitonin on blood pressure in the one-kidney hypertensive rat

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Summary

1. The tonin concentration of saliva and submaxillary glands was studied in one-clip hypertensive rats with or without the contralateral kidney.

2. Salivary tonin concentration was elevated in one-kidney hypertensive rats, but not in one-kidney normotensive or two-kidney, one-clip hypertensive rats. In contrast, an elevated submaxillary gland tonin concentration was found only in uninephrectomized animals, whether normotensive or hypertensive.

3. A single intravenous administration of rabbit tonin antiserum into one-kidney hypertensive rats restored blood pressure to normal in seven out of ten animals. There was little change in blood pressure in two-kidney, one-clip hypertensive, uninephrectomized or sham-operated rats.

4. These findings suggest a connection between the physiology of the kidney and of the submaxillary gland in the rat, and indicate that tonin may play a significant role in maintaining high blood pressure in one-kidney hypertensive animals.

Key words: antiserum injection, hypertension, renal artery constriction, saliva, submaxillary gland, tonin.

Introduction

An enzyme, tonin, which is present in different rat tissues, is capable of releasing angiotensin II from angiotensin I, or directly from a natural protein substrate (angiotensinogen) present in plasma or from the synthetic tetradecapeptide renin substrate (Boucher, Saidi & Genest, 1972; Boucher, Asselin & Genest, 1974). The physicochemical characteristics and substrate specificity of tonin have been described (Demassieux, Boucher, Grise & Genest, 1976; Schiller, Demassieux & Boucher, 1976). It has so far been impossible to make accurate measurements of the concentration of this enzyme in rat tissues except in the submaxillary gland of rats over 250 g in weight, and in rat saliva, because of the presence in rat plasma and tissues (Boucher et al., 1974) of a strong protein inhibitor. α-Adrenergic receptor stimulation enhances both tonin release into the saliva and tonin synthesis in the submaxillary gland, the former being inhibited by angiotensin II (Garcia, Boucher & Genest, 1976; Garcia, Kondo, Schölkens, Boucher & Genest, 1977). The fall in blood pressure after infusion of angiotensin II antibodies or of synthetic antagonists indicates that the renin–angiotensin system is involved in the pathogenesis of the experimental hypertension that results when the artery of one kidney is clamped ('two-kidney, one-clip Goldblatt hypertension') (Pals, Masucci, Denning, Sipos & Fessler, 1971; Brunner, Kirshman, Sealy & Laragh, 1971; MacDonald, Boyd & Peart, 1975). In contrast, the one-kidney Goldblatt model is associated with an increase of exchangeable sodium (Tobian, Coffee & McCrea, 1969) and a positive sodium balance (Swales, Thurston,
Queiroz & Medina, 1972). However, by no means every one-kidney animal develops a positive sodium balance (Swales et al., 1972), which suggests that both a pressor mechanism and sodium retention might contribute to the development of hypertension. Moreover, neither chronic restriction nor acute deprivation of dietary salt affects the development or maintenance of hypertension in rats with one clamped artery, whether the contralateral kidney is present or not (Thurston & Swales, 1976).

There may be an active factor other than renin in kidney extracts (Helmer, 1958; Kira, Ohnishi, Yamamoto, Konishi, Yamatori & Kamiguchi, 1971; Skeggs, Kahn, Levine, Dorer & Lentz, 1977). This finding, the uncertain role of the renin–angiotensin system in the pathogenesis of experimental hypertension, together with the observed effect of tonin on mesenteric arteries (Kondo, Garcia, Demassieux, Manku, Horrobin, Boucher & Genest, 1977) has induced us to investigate the behaviour of this enzyme in the two most widely used models of experimental hypertension.

Materials and methods

Hypertensive animals

Two-kidney, one-clip hypertension was produced in male Sprague–Dawley rats weighing 240–280 g by constriction of the left renal artery with a silver clip having an internal gap of 0·15–0·20 mm; the contralateral kidney was left untouched. All rats were given Purina rat chow and allowed free access to tap water. One-kidney hypertensive rats were similarly prepared and maintained, except that during surgery the contralateral kidney was removed.

Blood pressure was measured indirectly twice a week by means of a tail cuff under light ether anesthesia, and recorded on a Grass model 7 polygraph fitted with a 7P 8 preamplifier and a model 1010 Grass crystal microphone as a pulse detector. Two or three measurements were taken and the average was used. Rats were considered hypertensive when their systolic blood pressure was consistently 150 mmHg or higher during the 3 weeks before experiments were started.

Two additional groups of rats were used as control animals. The first was subjected to a sham operation in which the left kidney was exposed and the renal artery stripped of surrounding tissue; the second was subjected to a right nephrectomy. All experiments were done 4 weeks after the operation. Each group was divided into two: in one tonin was measured in saliva and submaxillary gland and in the second tonin antiserum was given.

Tonin concentrations in saliva and submaxillary gland

Animals were anaesthetized with sodium pentobarbital (Nembutal) (60 mg/kg intraperitoneally). A glass cannula was inserted into the oesophagus and connected to a 2 ml plastic vial. The volume of saliva collected during a 45 min period was calculated by weight difference and expressed in μl/min. Saliva secretion was stimulated with carbamylcholine chloride (25 μg intraperitoneally per rat) (Carbachol, Sigma), which increases the flow of saliva without increasing its tonin concentration (Garcia et al., 1976). The collected saliva was diluted ten times with borate/phosphate buffer, pH 7 (made by mixing sodium borate, 0·05 mol/l, with KH₂PO₄, 0·1 mol/l, to the desired pH). The submaxillary glands were then removed and homogenized with a Polytron homogenizer, the homogenate being diluted ten times with the same buffer containing 0·02% of a metal-free non-ionic detergent (Acationox, Scientific Products, Ill., U.S.A.) and 0·87% ethylenediaminetetra-acetic acid (EDTA) at pH 7. Both diluted saliva and submaxillary gland suspension were kept at −20°C until processed. Before processing, the sample containing the gland was centrifuged; the supernatant was harvested and further diluted 1:1000 with the EDTA/Acationox solution.

Tonin activity in saliva and submaxillary gland was measured fluorimetrically (Boucher et al., 1974) with angiotensin I as substrate (iso-5-angiotensin I, Schwarz/Mann, New York, U.S.A.; lot 2313). Some modifications have been introduced: EDTA (1·3 mmol/l) was included in the buffer in addition to dipyridyl (8 mmol/l) and di-isopropyl fluorophosphate (DFP) (0·70 mmol/l), and the incubation step was reduced to 10 min and incorporated into the Technicon analytical system. The substrate was introduced at the same time of sampling by a synchronized pump (Boucher, Demassieux, Garcia & Genest, 1977). The concentration of histidyl-leucine released was calculated by comparison of the fluorescence produced with that of standard solutions. Tonin concentration was expressed as either mol x 10⁻⁹ of His-Leu released from the substrate per ml of saliva, or per gram of submaxillary gland during a 10 min incubation period.
Effect of antiserum

A specific antiserum was raised in rabbits (J. Gutkowska, unpublished work) through a series of injections of highly purified tonin obtained from rat submaxillary gland (Demassieux et al., 1976). The animals were anaesthetized with sodium pentobarbital as described above, the jugular vein was cannulated for injection and the blood pressure was continuously recorded through a carotid artery catheter connected to a Statham pressure transducer. Undiluted antiserum from the same batch was then injected as a single dose of between 0.1 and 0.3 ml. No dose-response studies were done because of the scarcity of the material. Pooled normal rabbit serum was injected as a control.

All results are given as mean values ± SD. Comparisons were made by unpaired t-test.

Results

Tonin concentration in saliva and submaxillary gland

A systolic blood pressure of 108 ± 9 mmHg and 106 ± 8 mmHg was found in sham-operated and uninephrectomized animals respectively. The systolic blood pressure was 171 ± 8 mmHg for the two-kidney hypertensive rats and 179 ± 20 mmHg for the one-kidney hypertensive animals. The difference in blood pressure between the two hypertensive groups was not significant.

Although salivary tonin was significantly higher in the one-kidney hypertensive group than in any other, no change was observed in the two-kidney hypertensive group (Table 1). Uninephrectomy alone also induced a significant increase (P < 0.001) of tonin concentration in the submaxillary gland. Clamping of the renal artery did not affect this value either in uninephrectomized animals or in animals with both kidneys (Table 1).

Bilateral nephrectomy did not significantly change the tonin concentration of either saliva (28 ± 14 nmol in sham-operated, and 17 ± 8 nmol of His-Leu min⁻¹ ml⁻¹ in bilaterally nephrectomized rats) or submaxillary gland (20 ± 4 µmol in sham-operated and 27 ± 10 µmol of His-Leu min⁻¹ g⁻¹ of tissue in bilaterally nephrectomized rats) observed 24 h after surgery.

Effect of tonin antiserum

In the animals selected for investigation of the effect of tonin antiserum, no difference was observed in basal mean blood pressure either

![FIG. 1. Effect of tonin rabbit antiserum on the change in blood pressure (mean ±SD) in rats with one-kidney hypertension (○—○) or two-kidney hypertension (■—■) and in sham-operated (▼—▼) and uninephrectomized rats (▼—▼).](image)
mmHg (Fig. 1). Seven of ten one-kidney hypertensive rats reached a normal mean blood pressure (<125 mmHg) when 0-3 ml of antiserum was injected. None of the two-kidney hypertensive animals became normotensive with this dose. The fall in blood pressure was immediate and gradually returned to baseline values in the next 5 min. No such change was observed when normal pooled rabbit serum was injected, the maximal drop in mean blood pressure being 6 ± 5 mmHg for the one-kidney, and 4 ± 4 mmHg for the two-kidney, hypertensive rats when a dose of 0-3 ml was used. The change in mean blood pressure induced by injection of tonin antiserum into one-kidney hypertensive animals was highly significant (P < 0.001).

Discussion

In one-kidney hypertensive animals, the salivary concentration of tonin is higher than in normotensive control rats or in two-kidney hypertensive rats. The elevated tonin concentration in submaxillary glands in uninephrectomized animals, whether normotensive or hypertensive, suggests a connection between kidney and submaxillary glands which we cannot yet explain. Total nephrectomy did not affect gland or salivary tonin concentration, at least after 24 h.

The striking fall in blood pressure produced by the single injection of (rabbit) tonin antiserum in one-kidney hypertensive rats seems to be a specific effect, as no change was observed in animals injected with normal rabbit serum. Antitonin given to rats with two-kidney hypertension also lowered their blood pressure transiently, but modestly. This suggests that tonin may help to maintain high blood pressure in one-kidney hypertensive rats, but makes a trivial contribution to blood pressure in two-kidney hypertension.

We can only speculate on the relationship between the tonin concentration in saliva and its plasma concentration, which we cannot measure because of the presence of a protein inhibitor in plasma. However, the rapid fall in blood pressure in one-kidney hypertensive rats after injection of an antitonin antibody suggests that there is an elevated concentration of free tonin in these rats, or of tonin attached to arterial receptors in the circulation.

These observations are consistent with those of Kondo et al. (1977), who described both potentiation to the action of noradrenaline and a direct vasoconstriction when tonin was infused into the rat mesenteric preparation. These effects were not inhibited by an angiotensin II antagonist, which suggests either that tonin reacts with a protein substrate in the endothelial cell membrane, so as to generate angiotensin II in situ, or that it acts directly on the arterial wall.

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References


