**Tonin–angiotensin II system in hypertension**

R. BOUCHER, R. GARCIA, J. GUTKOWSKA, S. DEMASSIEUX AND J. GENEST

Clinical Research Institute of Montreal, Montreal, Canada

**Summary**

1. A single intravenous administration of rabbit tonin antiserum into one-kidney one-clip 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.

2. Infusion of tonin in control rats did not modify arterial blood pressure. However, in indomethacin salt-treated rats a marked increase in arterial blood pressure was observed under tonin infusion.

3. Plasma tonin activity was significantly increased in human essential and renovascular hypertension.

4. These findings strongly suggest that tonin is important in the maintenance of high blood pressure. However, other factors (possibly prostaglandins and sodium) have to be modified in order to activate the tonin–angiotensin II system.

Key words: antiserum injection, hypertension, noradrenaline, prostaglandins, renal artery constriction, sodium, tonin.

**Introduction**

Tonin is unique, among the proteases presently known, in its ability to hydrolyse angiotensin I, the renin tetradecapeptide renin-substrate and angiotensinogen to form angiotensin II rapidly and directly. In the circulation as well as in most tissues, its enzymic activity is inhibited by a protein. Tonin activity may be determined by a number of methods such as bioassay, chemical assay (using a fluorimetric technique for measurement of the His-Leu dipeptide), or by radio-immunoassay (Boucher, Saidi & Genest, 1972; Boucher, Asselin & Genest, 1974; Boucher, Demassieux, Garcia & Genest, 1977 a,b). Tonin has been purified to a state of homogeneity from rat submaxillary glands (Demassieux, Boucher, Grise & Genest, 1976) isolated from rat and human plasma (S. Demassieux, R. Boucher & J. Genest, unpublished work) and crystallized by Hayakawa, Kelly & James (1978).

Tonin potentiates the vasopressor effect of noradrenaline in the rat mesenteric artery preparation. In contrast to angiotensin I and to angiotensin II, this effect is not blocked by saralasin nor by the converting-enzyme inhibitors (Kondo, Garcia, Demassieux, Manku, Horrobin, Boucher & Genest, 1977). Further, it has been observed that a protein substrate is present in the perfused mesenteric arterial tissue.

Those observations strongly suggest the local formation of angiotensin II at sites inaccessible to endogenous angiotensin II, peptide antagonists to angiotensin II or inhibitors of converting enzyme. This alters our present concept which is based exclusively on the classical renin–angiotensin system and thus provides another unexpected pathway of action.

Three recent findings will be reported which substantiate a role of tonin in hypertension: 1, reversal of one-kidney hypertension in rat by antitonin; 2, effect of tonin on the blood pressure of the rat; 3, plasma tonin activity in patients with mild essential and renovascular hypertension.

**Methods and results**

*Reversal of one-kidney hypertension in rat by antitonin*

The fall in blood pressure after infusion of angiotensin II antibodies or of synthetic antagonist
suggests that the renin–angiotensin system is involved in the pathogenesis of the two-kidney one-clip Goldblatt hypertension, at least in the first 15 weeks (Masaki, Ferrario, Bumpus, Bravo & Khosla, 1977). In contrast, the one-kidney Goldblatt model does not respond to such treatments and does not appear to be maintained by the renin–angiotensin system. The mechanisms whereby chronic one-kidney hypertension develop are still unclear.

Studies from this laboratory have shown that blood pressure of rats with one-kidney one-clip hypertension can be lowered to normal levels by injections of antitonin (Garcia, Boucher, Gutkowska, Kondo, Demassieux & Genest, 1978). In these experiments, the mean blood pressure was the same for the hypertensive groups (178 ± 18 mmHg for the one-kidney and 176 ± 15 mmHg for the two-kidney hypertensive rats) and for the normotensive groups (111 ± 16 mmHg for the uninephrectomized and 100 ± 15 mmHg for the sham-operated rats).

A single injection of rabbit antiserum induced a dose-related fall in mean blood pressure with a maximum response of 51 ± 10 mmHg in one-kidney hypertensive rats. Seven out of ten one-kidney one-clip hypertensive rats reached a normal mean blood pressure (125 mmHg). The fall in blood pressure was immediate and gradually returned to baseline values in the next 5 min. In the two-kidney one-clip hypertensive animals antiserum administration induced a minimal depressor response of 4 ± 6 mmHg.

Measurement of plasma tonin activity by a solid phase radioimmunoassay, in the same groups of rats gave the following results: control animals (n = 10) 61 ng/ml ± 34 sd; in two-kidney one-clip hypertensive rats (n = 9) 192 ng/ml ± 73 sd; and in one-kidney one-clip hypertensive rats (n = 9) 342 ng/ml ± 122 sd.

These findings suggest that tonin may help to maintain high blood pressure in one-kidney hypertensive rats and could be implicated in the two-kidney hypertensive rats when hypertension enters the chronic phase (>15 weeks).

**Effect of tonin on rat blood pressure**

Intravenous infusion of amounts up to 15 μg of tonin/kg/min in normal rats had no effect on blood pressure. We know that vasoconstrictor action of pressor hormones is modulated by prostaglandins (Leary, Ledingham & Vane, 1974; Aiken & Vane, 1973; Malik & McGiff, 1974). Increase of pressure caused by renal artery constriction was significantly greater in animals receiving indomethacin, a known inhibitor of prostaglandin synthesis (Pugsley, Beilin & Peto, 1976; Romero & Strong, 1977). Indomethacin has also been shown to induce arterial hypertension in rats on a high salt intake (Sokolova, Nekrasova, Levitskaya, Speranskaya & Volkov, 1977).

The present study was designed to examine the effects of the administration of indomethacin, on the blood pressure response to tonin. Indomethacin (3 mg/kg/day) was given orally for 3 weeks to a group of rats, weighing 250–300 g. The drinking water was replaced by a 1% NaCl solution.

A typical blood pressure response curve to tonin in indomethacin-treated rats is shown in Fig. 1. In rats receiving indomethacin, infusion of tonin increased blood pressure. The increase in blood pressure lasted for the duration of the infusion. Sar⁴-Ile⁸-angiotensin II (10 mg/kg/min) was without any effect on the action of tonin, though the pressor action of angiotensin II was completely blocked by this angiotensin II antagonist. In contrast antibody to tonin lowered blood pressure to normal values.

**Tonin activity in patients with mild essential and renovascular hypertension**

The accurate measurement of true plasma tonin activity cannot be achieved at present because of the presence of a strong protein inhibitor in human plasma. However, preliminary studies showed that when human plasma is incubated with angiotensin I as substrate in the presence of converting enzyme, chymotrypsin and angiotensinase inhibitors, the angiotensin I is measured by a specific radioimmunoassay (J. Gutkowska, R. Boucher & J. Genest, unpublished work). To what extent the values obtained represent total plasma tonin activity remains to be evaluated.
Measurement of plasma tonin activity in peripheral venous blood was performed on plasma collected in chilled Vacutainer tubes (12 mg of EDTA/10 ml). Plasma can be frozen and stored before use. The phosphate buffer (0-2 M, pH 6-8) used, contains 5 mM-disodium EDTA, 3-4 mM-hydroxyquinoline; 0-2% dipyridyl; 0-1 mM-diisopropyl phosphorofluoridate and 0-25% bovine serum albumin. The ratio plasma/buffer was one to two.

Plasma (10 μl) was incubated at 37°C at pH 6-8 for 30 mins. The angiotensin activity was 1.1 pg/ml/min and those with renovascular hypertension had levels significantly higher than those of the normotensive controls.

Discussion

The most surprising observation was that administration of tonin to rats does not change the blood pressure. However, the finding that tonin appears to be responsible for the maintenance of elevated blood pressure in one-kidney one-clip hypertensive rats, suggests that unknown factors have to be modified in the normal animals for the activation of the tonin-angiotensin II system.

The increase in blood pressure with tonin infusion in indomethacin salt-treated rats, as well as the potentiation of the vasopressor effect of noradrenaline in the rat mesenteric artery preparation strongly suggest that PG and sodium are major elements for activation of tonin and local formation of angiotensin II within the walls of blood vessels.

No decrease in blood pressure was observed, with an antagonist to angiotensin II, during infusion of tonin in indomethacin salt-treated rats. This is in agreement with our previous observation in the mesenteric artery preparation (Kondo et al., 1977) and with the accepted finding that peptide competitive antagonist of angiotensin II does not affect the blood pressure in the one-kidney model of hypertension in rats.

The increase in plasma tonin activity in human essential and renovascular hypertension is exciting and favours a participation of the tonin–angiotensin II system in human hypertension.

These studies demonstrate that under conditions which are not yet well defined, tonin will form angiotensin II at vascular sites different from angiotensin II receptors. The increase in blood pressure may also occur through potentiation of the vasopressor effects of noradrenaline.

It appears reasonable to postulate that there is an interplay between sodium, noradrenaline, prostaglandins and the tonin–angiotensin II system and that their lack of balance may be involved in the mechanism of hypertension.

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