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Reduction of Hypertension in One-Kidney One-Clip Rabbits by Immunization with Tonin (40658)

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Tonin, an enzyme of the serine–protease family, is present in various rat tissues, especially submaxillary glands and kidneys. It releases angiotensin II directly from angiotensinogen, from the synthetic tetradecapeptide renin substrate and from angiotensin I (1, 2). This new enzyme has been purified and its physicochemical characteristics and substrate specificity are well established (3, 4). It has been crystallized by Hayakawa *et al.* (5).

The fall in blood pressure after adiministration of angiotensin II antibodies or angiotensin antagonists (saralasin) seems to indicate that the renin–angiotensin system is involved in the pathogenesis of the two-kidney, one-clip Goldblatt hypertension (one renal artery clamped, contralateral kidney untouched) (6–8).

The direct role of the renin–angiotensin system in inducing or maintaining elevated blood pressure in the experimental one-kidney one-clip Goldblatt hypertension (one renal artery clamped, contralateral nephrectomy) remains a highly controversial subject. Active immunization against angiotensin II was shown to be ineffective in preventing the development of this type of hypertension in rabbits (9, 10). Neither the administration of antirenin serum (11), angiotensin II synthetic antagonist (12), nor converting enzyme inhibitors (13) have been effective in lowering the blood pressure in this model of experimental hypertension.

Studies from our laboratory (14) have demonstrated that the blood pressure of rats with one-kidney one-clip hypertension can be decreased to normal levels by passive immunization with rabbit tonin antibodies. These recent findings, together with the availability of pure tonin, led us to investigate the effect of active immunization with this enzyme in the one-kidney one-clip hypertensive rabbit.

Materials and methods. Male New Zealand white rabbits (2 to 2.5 kg) were kept in a constant-temperature room, fed with Purina rabbit chow, and allowed free access to tap water.

After a control period of 1 week, the right kidney was removed under anesthesia (sodium pentobarbital, Nembutal, 30 mg/kg i.v.) through a flank incision.

One week later the left renal artery was constricted with a silver clip (15) having an internal gap of 0.58 mm. Blood pressure was measured at room temperature (22°) twice a week, indirectly in the central ear artery using the capsule of Grant and Rothschild (16). The average of three readings was recorded.

The animals were considered hypertensive when blood pressure was consistently at least 25 mm Hg higher than control values.

Eleven rabbits with hypertension of 3–5 weeks' duration were injected subcutaneously at several sites with a single dose of 50 μg of purified rat tonin (3). Tonin was diluted in 0.5 ml of 0.9% NaCl and well mixed with an equal volume of Freund's complete adjuvant. Blood was withdrawn every 2 weeks.

Serum antitonin was measured by radioimmunoassay (17). The incubation mixture consisted of 100 μl of ^{125}I-labeled tonin containing about 6000 cpm and 100 μl of antiserum at 1:1000 dilution.

The tubes were incubated at 4° for 24 hr. The antibody-bound tonin was precipitated by adding 50 μl of goat anti-rabbit γ-globulin diluted 1:50 in normal rabbit serum 1:1000 and incubating for 48 hr at 4°. The precipitates were isolated by centrifugation at 4000 rpm for 20 min at 4° and their radioactivity was determined in a gamma counter.

Serum antitonin was expressed as the percentage of bound labeled tonin.

Plasma renin activity was measured by radioimmunoassay of generated angiotensin I

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(18). The data are expressed as mean values ± SD. Comparisons were made by the paired or unpaired *t* test.

Results. The effect of immunization with 50 μg of purified tonin on rabbit No. 52 is shown in Fig. 1. After right nephrectomy and left renal artery constriction blood pressure rose till sustained levels were reached. Four weeks after the rabbit became hypertensive, tonin was injected. One week later the blood pressure has fallen and it attained preclamping values 2 weeks after the injection. Antibodies against tonin were detectable 20 days after immunization; they reached their highest titer 80 days later and gradually decreased thereafter. We could not use more concentrated plasma to increase the sensitivity of our assay for antitonin antibodies because of the presence in plasma of an interfering nonimmune protein which binds tonin. This may account for the discrepancy in time between the reduction in blood pressure and the detection of antibodies. Blood pressure remained below preimmunization levels throughout the observation period, increasing slightly 60 days after immunization. There was no apparent correlation between antibody titer and decrease in blood pressure.

Figure 2 relates to rabbit No. 84, in which the tonin injection did not elicit antibody formation. Blood pressure continued to rise after tonin injection in this case. Two groups of animals falling into the two different patterns, were clearly defined (Table I). Out of 11 hypertensive rabbits with a blood pressure of 83 ± 7 mm Hg injected with tonin, 5

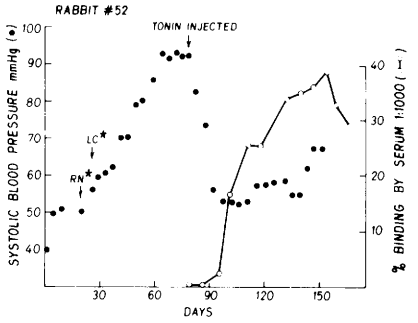

FIG. 1. Effect of the injection of tonin on blood pressure in a one-kidney, one-clip hypertensive rabbit (No. 52). RN = right nephrectomy, LC = left renal artery clipped. (●) BP, average of three readings; (○) antibody titer.

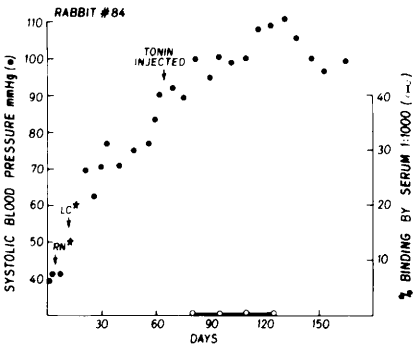

FIG. 2. Effect of the injection of tonin on blood pressure in a one-kidney, one-clip hypertensive rabbit (No. 84). Abbreviations and symbols as in Fig. 1.

responded with antibody formation (responders) and in all of these the blood pressure had decreased significantly at the end of 4 weeks from 86 ± 5 mm Hg to 60 ± 9 mm Hg ($P < 0.001$). In the second group, in which tonin injection was not followed by antibody formation (nonresponders), the blood pressure further increased over the corresponding period from 82 ± 9 mm Hg to 95 ± 8 mm Hg ($P < 0.001$). No significant differences were found between responders and nonresponders for the comparison between control and preimmunization blood pressures, but the difference was highly significant ($P < 0.001$) for the comparison 4 weeks and 3 months after immunization.

At the end of an observation period of 5 months the blood pressure in the responder group was further, although not significantly, reduced to 55 ± 10 mm Hg (not significantly different from controls), while in the nonresponder group it increased to 116 ± 13 mm Hg ($P < 0.001$).

No difference was found in plasma renin activity (PRA) 4 weeks after tonin injection between both the two groups: 2.13 ± 0.9 ng AI/ml·hr^{-1} in responders compared to 1.92 ± 1.3 ng AI/ml·hr^{-1} in nonresponders.

Discussion. These observations show that one-kidney one-clip hypertensive rabbits are clearly separated into two groups after being injected with tonin. The fall in blood pressure produced in five animals coincided with the appearance of antitonin antibodies; in none of the animals in which antibodies were not detected was the blood pressure lowered. The normalization of the blood pressure in the responder group lasted throughout the period

TABLE I. EFFECT OF ACTIVE IMMUNIZATION WITH TONIN ON ONE-KIDNEY, ONE-CLIP HYPERTENSIVE RABBITS

	Before immunization		After immunization			
Rabbit	Control BP (mm Hg)	BP before injection (mm Hg)	At 4 weeks BP (mm Hg)	Ab titer %	At 3 months BP (mm Hg)	Ab titer %
51	36	79	53	8	41	57
52	48	93	55	17	55	29
55	56	82	59	5	67	20
56	60	86	60	9	61	40
63	53	88	75	24	53	51
Mean ± SD	51 ± 9	86 ± 5	60 ± 9		55 ± 10	
84	39	81	91	0	111	—
86	34	72	81	0	111	—
87	49	98	104	0	129	—
91	47	80	95	0	99	—
94	44	80	99	0	130	—
6	41	78	102	0	—	—
Mean ± SD	42 ± 6	82 ± 9	95 ± 8		116 ± 13	

during which antibodies were being produced. This strongly suggests that tonin may be responsible for the development and maintenance of hypertension in one-kidney one-clip rabbits.

These observations are consistent with the fall in blood pressure observed in one-kidney one-clip hypertensive rats after they had been injected with antitonin antibody (14) and with the results of Kondo *et al.* (19), who described both potentiation to norepinephrine and a direct vasoconstriction when tonin was infused into the rat mesenteric preparation. These effects were not blocked by a synthetic angiotensin II antagonist, which suggests that tonin acts either upon a protein substrate in the endothelial cell membrane to generate angiotensin II *in situ*, or directly on the arterial wall. These findings could explain the lack of effect of angiotensin II antibodies or of angiotensin II antagonists in the one-kidney one-clip hypertensive animals (9–13).

It is interesting to note that a significant decrease in blood pressure in renovascular hypertensive animals has been reported after the injection of crude kidney homogenates (20–23).

Summary. Male New Zealand white rabbits with one-kidney one-clip Goldblatt hypertension of 3–5 weeks' duration were injected with purified tonin. Of 11 injected animals, 5 developed antibodies and in all of these animals blood pressure returned to normal levels. No change in blood pressure was observed in 6 animals in which no antibodies were detected. These findings are consistent with previous studies in rats and suggest that tonin plays a significant role in maintaining high blood pressure in one-kidney hypertensive animals.

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