The Effect of Nephrectomy and of Renomedullary Extracts on the Blood Pressure of Experimentally Hypertensive Rabbits[1] (36523)

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When the renal artery to one kidney of a rabbit is clamped, the blood pressure rises; when the contralateral kidney is subsequently removed a further elevation frequently occurs. The mechanism of the hypertension following the second operation has been the subject of controversy. Pickering (1) divided this chronic hypertensive state into two phases: an early one lasting at least 1 week, in which cure could be obtained by excising the clamped kidney, and a second phase, some weeks later during which such a procedure was not curative at least during the brief period of survival in the renoprival state. Since the renin source was eliminated by nephrectomy, he did not believe it could be the cause of the late phase of chronic "one-kidney" Goldblatt hypertension.

An alternate hypothesis, that blood pressure elevation results from the loss of a renal antihypertensive factor, was proposed by Grollman, Harrison and Williams (2) and has been supported by the studies of Muirhead, Jones and Stirman (3), who were able to isolate an antihypertensive principle from renal medullary tissue.

The purpose of this study was to repeat Pickering's observations in a shorter experimental protocol and to investigate the effects of renomedullary extracts on the blood pressure during various phases of experimental hypertension in the rabbit.

Materials and Methods. I. Rabbit techniques and protocols. The studies were performed in 40 male New Zealand white rabbits weighing from 1.8 to 3.0 kg and maintained on standard rabbit pellets (Purina) and tap water. Arterial blood pressure was measured indirectly in the central ear artery, using the Grant–Rothschild capsule (4) and after 10 min in a room kept at 37°. Surgical procedures were performed under anesthesia produced by pentobarbital (40 mg/kg) atropine sulfate (0.13 mg/kg) and pentylenetetrazol (Metrazol, 48 mg/kg), the last added to maintain respiration. The mixture was injected slowly through the marginal ear vein. Operative procedures included nephrectomy and renal artery constriction, which was achieved by means of a silver clamp, internal diameter 0.7 mm, or 0.8 mm for rabbits weighing 2.3 kg or more. Renomedullary extracts (RME) or prostaglandin solutions, prepared as described below, were injected intraperitoneally in 4 ml doses through an 18-gauge needle.

A. Experiments on the effect of the removal of the ischemic kidney on blood pressure. Twelve rabbits were observed for 18 days, during which control blood pressures were determined every other day. The left renal artery was then constricted as described above. After a further 30-day period of blood pressure observation, the right kidney was removed and the animals were divided into two groups. In the first group (5 animals) the remaining ischemic left kidney was removed after 7 days; in the second group (7 animals), after 30 days. After nephrectomy, the blood pressure was measured every hour for 9 hr and at 24 hr in both groups.

B. Experiments with renomedullary extracts and prostaglandins. After a shorter control period of 2–7 days, 28 rabbits were subjected to left renal artery constriction. Blood pressures were determined every other day for 14 days. Then these animals were divided into three groups:

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Group 1. Nine rabbits purposely selected for elevated blood pressure following clamping only, were injected with RME or boiled RME, as described below, 4 ml/day for 3 consecutive days. Blood pressure was measured every 2 hr for 8 hr after the first injection, then on the second and third day just prior to reinjection, and for several days thereafter. Rectal temperature was measured, just before placing the rabbits in the 37° room, during the injection series.

Group 2. Nineteen rabbits were subjected to contralateral (right) nephrectomy 14 days after left renal artery constriction, and the temperature and blood pressure were measured every other day for 7 days. Then they received 4 ml/day of either RME, boiled RME, prostaglandin A_1 or prostaglandin E_2 for 3 consecutive days, with blood pressure and temperature measurements as in Group 1.

Group 3. Six rabbits, survivors of Groups 1 and 2, were injected with 4 ml/day of RME for 3 consecutive days beginning 30 days after contralateral nephrectomy. Blood pressure and temperature measurements were performed as for Groups 1 and 2.

II. Preparation of renomedullary extracts (RME). Fifty to 100 g of individually frozen rabbit kidney medullas[2] were mixed with half their weight of 0.9% saline solution and thawed at room temperature. The mixture was then disrupted for 60 sec in a Servall Omni-Mixer. The suspension was placed in a beaker along with another 0.5 vol of saline solution used to rinse the blender cups and blades. The mixture was then centrifuged at room temperature, 30 min at 1500*g* and the supernatants were decanted and recentrifuged for 3–4 hr at 18,800*g* at 4°. The clear ruby-red supernatant ("RME") was aspirated carefully from the centrifugate and frozen in 5 ml aliquots. Alternately, the extract was adjusted to pH 5.1 with 1 *N* HCl and heated in boiling water for 5 min, then centrifuged at room temperature, 1500*g* for 30 min. The clear, straw-colored supernatant ("boiled RME") was also frozen in 5 ml aliquots. Four milliliters of the final extract is derived from 2 g of rabbit medulla which is approximately the amount contained in one rabbit kidney.

III. Prostaglandin solution. Prostaglandins A_1 and E_2, kindly supplied by Dr. Jack Hinman of the Upjohn Company, were dissolved in 0.1 ml of ethanol and 0.9 ml of Na_2CO_3, 20 mg/100 ml, per each milligram; further dilutions were made with 0.9% saline solution. These solutions were administered intravenously to pentobarbital-anesthetized, pentolinium-treated normal male Sprague-Dawley rats, and the concentrations were adjusted to make their acute dilator action equal to that of an equal volume of boiled RME. By this method the doses given were 24 μg/kg of PGA_1 and 12 μg/kg of PGE_2. Two rabbits received a larger dose (40 μg/kg) of PGA_1 and one received a larger dose (24 μg/kg) of PGE_2.

Results. Effect of removal of sole ischemic kidney on the blood pressure. The average blood pressure of these rabbits rose slowly from about 65 to 85 mm Hg after renal artery clamp alone; it rose sharply to approximately 120 mm Hg after contralateral nephrectomy. Removal of the ischemic kidney 7 days after that nephrectomy caused a return to near normal blood pressure values in 9 hr; at 24 hr the average blood pressure was 72 mm Hg. No significant fall in blood pressure occurred when the sole ischemic kidney was removed 30 days after contralateral nephrectomy. The blood pressure at 24 hr was 110 mm, clearly still hypertensive.

Effect of renomedullary extracts at various stages of experimental hypertension. In the rabbits to be treated with renomedullary extracts, the mean blood pressure rose in a manner comparable to that described above after the various operative procedures. Group 1 animals were selected for relatively high blood pressures 14 days after clamping. Their blood pressure was not affected by the renomedullary extract, nor in the control experiment using boiled extract (Fig. 1). Figure 2 shows that the mean blood pressure of Groups 2 and 3 were further elevated after contralateral nephrectomy. In Group 2, when RME was injected 7 days after contralateral

[2] Obtained from Pel-Freez Biologicals, Rogers, AR, where the medulla was separated at sacrifice and immediately frozen for shipment.

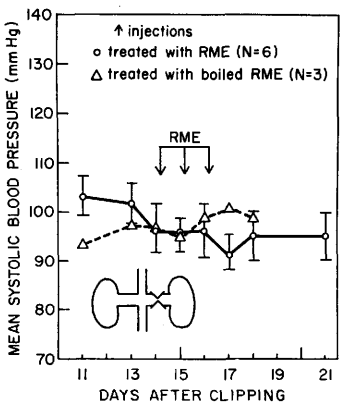

FIG. 1. Effect of renomedullary extracts on the blood pressure of rabbits hypertensive 14 days after unilateral renal artery constriction.

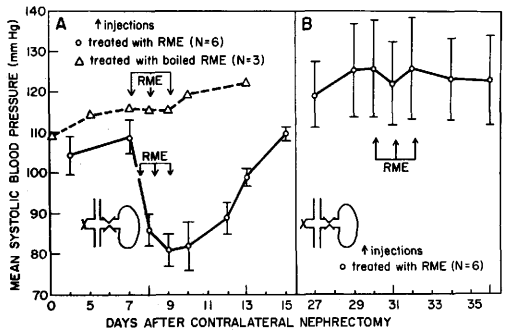

FIG. 2. Effect of renomedullary extracts on the blood pressure of rabbits hypertensive: (A) 21 days after unilateral renal artery constriction and 7 days after contralateral nephrectomy, (B) 44 days after unilateral renal artery constriction and 30 days after contralateral nephrectomy.

nephrectomy (Fig. 2A), the blood pressure fell to near normal values and recovered slowly after the series of three injections, reaching previous values 6 days after the administration of the last dose of extract; there was no change in the blood pressure of the three rabbits treated with boiled RME.

The fall in blood pressure observed in the Group 2 rabbits treated with RME was studied more closely in four rabbits presented in Table I. As shown, the blood pressure decline first became manifest 8 hr after RME injection. No changes in rectal temperature were observed in any of the groups.

In Group 3, injected 30 days after contralateral nephrectomy (Fig. 2B), no fall in blood pressure was observed. The large standard error in the mean blood pressure of this group reflects the fact that two subgroups appeared in this phase of hypertension, some rabbits developing mild hypertension (100–120 mm) while others had severe hypertension (140–170 mm). Nevertheless, none of the animals showed any response to the RME injections.

TABLE I. Changes in Systolic Blood Pressure of Rabbits Treated with RME[a] 7 Days After Contralateral Nephrectomy.

Rabbit	BP Preinjection	Change in BP; after injection (hr): 2	4	6	8	24
1	120	−2	−2	−10	−20	−40
2	106	+2	−1	—	−8	−31
4	120	—	0	—	−10	−35
C3	112	−7	−10	−14	−20	−30

[a] Renomedullary extract.

Effect of prostaglandins A_1 and E_2. Since RME lowered the blood pressure only in the Group 2 rabbits, prostaglandin solutions were given in this same period. Figure 3 shows that the blood pressure of these rabbits was not changed over the 3-day injection period. Although transient changes were observed in some of the rabbits when the blood pressure was studied in detail (Table II), no prolonged changes were seen.

Discussion. It should be noted that Pickering (1) used a slightly different procedure. He first excised one normal kidney and then clamped the renal artery of the opposite side. He waited 7–9 weeks before removing the sole remaining ischemic kidney. Since in a previous study (5) we had made relatively important observations on plasma renin levels using a different protocol (clamping one renal artery first, then removing the opposite kidney), it was decided to adopt this procedure but to observe the effect on the blood pressure of excising the clamped kidney early and late after the establishment of "one-kidney" hypertension. Removal of the sole ischemic kidney normalized the blood pressure if performed at 7 days after contralateral nephrectomy but not at 30 days.

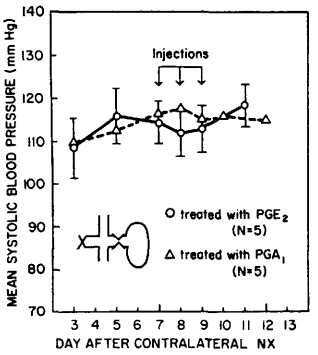

FIG. 3. Effect of prostaglandins on the blood pressure of rabbits hypertensive 21 days after unilateral renal artery constriction and 7 days after contralateral nephrectomy.

Thus two phases of one-kidney Goldblatt hypertension can be distinguished, an "early" phase when the blood pressure responds to removal of the clamped kidney and a "chronic" phase when it does not.

Early phase. This phase is further characterized by an elevated plasma renin activity (PRA) and renin substrate levels, as reported by this laboratory (5) for the rabbit and by Blair-West *et al.* (6) for the sheep. The hypertension is curable by injections of renal medullary extracts (RME). The data suggest that a pressor secretion of the ischemic kidney may be responsible for the rise in blood pressure in this phase, and that RME may counteract this effect.

Chronic phase. This phase is defined by the fact that the removal of the sole ischemic kidney does not lower the blood pressure. This was first described for the rabbit by Pickering and herewith confirmed by us. Similar observations have been made in the rat (7, 8). Furthermore, there is no accompanying pressor activity in the circulating blood (9); PRA and renin substrate are normal (5); and the hypertension does not respond to acute intravenous injection of antirenin antibodies (10), nor to renal medullary extracts as reported herewith. Hence the renal pressor system does not seem to be responsible for one-kidney hypertension in this last phase. However, the blood pressure can be normalized by restoring kidney function, either by transplant (11) or by removing the clip (12–14). This phenomenon could be explained on the basis of restored excretory function (14) or by the restoration into the circulation of a renal antihypertensive principle (15–18). The relative importance of these mechanisms in controlling the blood pressure has been particularly difficult to assess due to the interdependence of their actions. But it is clear that as the period after contralateral nephrectomy is extended, the one-kidney hypertensive state becomes increasingly more fixed. This relative irreversibility could be an explanation for the observation that the blood pressure in this phase was not lowered by the same brief treatment with RME that was effective in the early phase, or the unresponsiveness could be attributable

TABLE II. Changes in Systolic Blood Pressure of Rabbits Treated with Prostaglandins 7 Days After Contralateral Nephrectomy.

Rabbit	Injection (μg/kg)	BP Preinjection	Change in BP; after injection (hr): 1	2	3	6	8	24
a	PGA_1, 24	122	−2	+3	−2	−2	−2	−2
c	PGA_1, 24	120	−22	−2	0	+2	0	+2
d	PGA_1, 24	100	−20	+10	+5	+2	+5	+5
E4	PGA_1, 40	118	−35	−20	+2	+2	—	+2
E5	PGA_1, 40	123	−31	−13	−3	+2	—	−3
1	PGE_2, 12	125	0	−5	−3	−5	−5	−5
2	PGE_2, 12	118	+2	0	−3	−15	−8	−3
3	PGE_2, 12	106	−2	0	+2	−6	−10	−2
4	PGE_2, 12	124	−4	—	—	—	−1	+1
5	PGE_2, 24	100	—	0	—	—	0	−5

to the fact that the RME acts only in the presence of elevated plasma renin.

We also observed that RME was not effective in lowering the blood pressure of unilaterally clamped rabbits with the contralateral kidney present. In this preparation in our previous study (5) no increase in renal pressor activity was observed.

Nature and mode of action of renal medullary extracts. Since the material used in this study was a crude extract of the whole medulla, we cannot further compare it with the several antihypertensive products which have been isolated from the kidney (18–22), any of which might account for the activity observed. PGA_1 or PGE_2 may be excluded because they are heat stable, while the RME was inactivated by heating. Furthermore, neither PGA_1 nor PGE_2 given in amounts presumably present in the renal medullary extract[3] could duplicate its action. Perhaps, however, a thermolabile substance could act to potentiate the action of prostaglandins.

The possibility that nonspecific agents such as ADP (24) or pyrogens (25) could account for the action seems eliminated by the lack of effectiveness of RME in the first and third periods. The special responsiveness of the early phase of one-kidney hypertension to renal medullary extracts seems to be mediated indirectly since direct vasodepressors would be expected also to be antihypertensive at other stages of hypertension. The fact that this is the only phase when the renal pressor system is overactive might suggest that the extracts interfere with that system; there is some evidence favoring this hypothesis (15, 26).

Alternatively it may be postulated that a generally more labile status of the blood pressure exists in the early phase and may render the organism especially susceptible to RME, a finding also reported by Milliez *et al.* (27). Perhaps an increased dosage or a longer period of treatment would have shown RME to be effective in a later hypertensive phase as well.

The special usefulness of these findings is to indicate that there is a phase in experimental hypertension when the blood pressure is especially sensitive to the antihypertensive action of renal medullary extracts. Although other workers have described hypotensive effects of certain extracts derived from renal tissue (18–23), there has been no attention given to the phase of hypertension in which the extracts have been tested. At the onset of "one-kidney" hypertension the mechanisms for maintaining the blood pressure seem to be changing. Not only is an elevated plasma renin found, but the condition is susceptible to cure by nephrectomy and to normalization of blood pressure with renal medullary extracts. These three conditions do not apply to the later phase of chronic one-kidney hypertension. We believe that the mechanisms for maintaining the blood pressure in the early and late stages of one-kidney hypertension deserve further study.

Summary. In 40 male rabbits, the course of experimental renal hypertension was studied at three points in its development: (a) at 14 days after unilateral renal artery constriction, (b) at 7 days after contralateral nephrectomy, and (c) at 30 days after this nephrectomy. The removal of the sole ischemic kidney normalized the blood pressure when performed at point (b) but had no effect when performed at point (c). Crude extracts prepared from rabbit kidney medulla normalized the blood pressure when injected intraperitoneally for three days at point (b) but had no effect when injected at points (a) and (c). The active agent in the extract was thermolabile, and its action was not duplicated by a similar dose of prostaglandins A_1 or E_2. An attempt is made to characterize and differentiate the second and third phase and the effect of renomedullary extracts in terms of the interaction of the renal pressor

[3] Prostaglandins in larger doses (1–2 mg/kg/day, ip) have antihypertensive effects; the present dose (0.012–0.040 mg/kg) had only a transient early depressor action (Table II) quite unlike the delayed and greater effect of RME. The amount to be injected was chosen because when given intravenously to the rat it had a similar hypotensive effect to that of the renal medullary extract used in this study. Furthermore, this amount of prostaglandin has been reported to be present in 2 g of rabbit medulla according to the studies of Daniels *et al.* (23).

and antihypertensive systems.

1. Pickering, G. W., Clin. Sci. **5**, 229 (1945).

2. Grollman, A., Harrison, T. R., and Williams, J. R., Jr., Amer. J. Physiol. **139**, 293 (1943).

3. Muirhead, E. E., Jones, F., and Stirman, J. A., J. Lab. Clin. Med. **56**, 167 (1960).

4. Grant, R. T., and Rothschild, P. A., J. Physiol. (London) **81**, 265 (1934).

5. Romero, J. C., Lazar, J. D., and Hoobler, S. W., Lab. Invest. **22**, 581 (1970).

6. Blair-West, J. R., Coghlan, J. P., Denton, D. A., Orchard, E., Scoggins, B. A., and Wright, R. D., Endocrinology **83**, 1199 (1968).

7. Floyer, M. A., Clin. Sci. **14**, 163 (1955).

8. Liard, J. F., Experientia **25**, 934 (1969).

9. Blaquier, P., Bohr, D. F., and Hoobler, S. W., Amer. J. Physiol. **198**, 1148 (1960).

10. Weiser, R. A., Johnson, A. G., and Hoobler, S. W., Lab. Invest. **20**, 326 (1969).

11. Gomez, A. H., Hoobler, S. W., and Blaquier, P., Circ. Res. **8**, 464 (1960).

12. Blackett, R. B., and Sellers, A. L., Clin. Sci. **10**, 177 (1951).

13. McDonald, J. G., Louis, W. J., Renzini, V., Boyd, G. W., and Peart, W. S., Circ. Res. **27**, 197 (1970).

14. Liard, J. F., and Peters, G., Experientia **26**, 743 (1970).

15. Sen, S., Smeby, R. R., and Bumpus, F. M., Circ. Res. **21** (Suppl. II), II-129 (1967).

16. Muirhead, E. E., Stirman, J. A., and Jones, F., J. Clin. Invest. **39**, 266 (1960).

17. Hickler, R. B., Birbari, A. E., Kamm, D. E., and Thorn, G. W., *in* "L'Hypertension Artérielle" (P. Milliez and P. Tcherdakoff, eds.), p. 188. L'Expansion Scientifique Francaise, Paris (1966).

18. Milliez, P., Lagrue, G., Meyer, P., Devaux, C., and Alexandre, J. M., *in* "L'Hypertension Artérielle" (P. Milliez and P. Tcherdakoff, eds.), p. 203. L'Expansion Scientifique Francaise, Paris (1966).

19. Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fowts, P. J., and Kempf, G. F., J. Exp. Med. **73**, 7 (1941).

20. Hamilton, J. G., and Grollman, A., J. Biol. Chem. **233**, 528 (1958).

21. Muirhead, E. E., Daniels, E. G., and Hinman, J. W., *in* "L'Hypertension Artérielle" (P. Milliez and P. Tcherdakoff, eds.), p. 177. L'Expansion Scientifique Francaise, Paris (1966).

22. Lee, J. B., Gougoutas, J. Z., Takman, B. H., Daniels, E. G., Grostic, M. F., Pike, J. E., Hinman, J. W., and Muirhead, E. E., J. Clin. Invest. **45**, 1036 (1966).

23. Daniels, E. G., Hinman, J., Leach, B. E., and Muirhead, E. E., Nature (London) **215**, 1298 (1967).

24. Gordon, D. B., Amer. J. Physiol. **196**, 1340 (1959).

25. Page, I. H., Taylor, R. D., and Corcoran, A. C., *in* "Hypertension" (E. T. Bell, ed.). Univ. of Minnesota Press, Minneapolis (1951).

26. McGiff, J. C., Terragno, N. A., Crowshaw, K., and Lonigro, N., Clin. Res. **18**, 510 (1970).

27. Milliez, P., Lagrue, G., Meyer, P., and Boivin, P., Pathol. Biol. **11**, 184 (1963).

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