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Renal Pressure–Flow Relationships in Severely Hypertensive Rabbits (40903)[1]

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Abstract. The relationship between renal perfusion pressure and renal blood flow (RBF) was studied in the contralateral or not-clipped kidneys of seven two-kidney one-clip renovascular severely hypertensive rabbits and in the right kidneys of eight normotensive rabbits. Thirty to forty days after clipping the left renal artery, severely hypertensive rabbits were characterized by blood pressures significantly greater than normotensive (105 vs 80 mm Hg), and plasma renin activities three times greater than in normotensive rabbits. The absolute RBF to the right kidney was greater in hypertensive rabbits, but these kidneys were hypertrophied so that renal blood flow per gram kidney weight was the same (2.4 ml/min) in kidneys of both hypertensive and normotensive rabbits. Sequentially reducing renal perfusion pressure decreased RBF similarly in both groups suggesting hypertensive rabbits required 27% greater perfusion pressure to achieve RBF similar to that of normotensive rabbits. When the competitive angiotensin antagonist [Sar^1-Ile^8]-angiotensin II was administered to both groups, there was no significant change in the RBF of normotensives. In the hypertensive rabbits, however, blood pressure dropped to a value not different from normotensive while renal vascular resistance decreased such that RBF was maintained at a normal level. These results suggest the contralateral kidneys of severely hypertensive rabbits chronically adapt RBF to normal levels despite elevated renal perfusion pressure.

Severe renovascular hypertension is one of two distinct forms of high blood pressure (BP) which is evoked by partial occlusion of one renal artery (1–4). It is characterized by a combination of very high BP and high plasma renin activity (PRA) (1, 2, 5) and a depletion of the extracellular fluid volume (2, 4). This is believed to derive from a pressure related natriuresis via the exposed, contralateral kidney which is not clipped (1, 2, 4). It has been suggested (1) that the pressure diuresis of severe renovascular hypertension is a function of the contralateral kidney's inability to adapt to severely elevated perfusion pressures. However, the fact that the animals remain alive despite this loss of fluid volume suggests they may have achieved some temporary new equilibrium state between elevated pressure and renal fluid loss.

This study was undertaken to determine whether, in a state of severe renovascular hypertension, the contralateral kidney is able or unable to adapt its blood flow to the severely elevated perfusion pressure. Furthermore, since this form of renovascular hypertension is characterized by such a high PRA, use of the competitive angiotensin antagonist, [Sar^1-Ile^8]-angiotensin II, was employed to determine the influence of the elevated circulatory angiotensin levels in the relationship between renal perfusion pressure and blood flow.

Materials and methods. Experiments were performed on two groups of adult male New Zealand white rabbits weighing 1.5 to 3.5 kilograms, maintained on a normal rabbit chow diet (Purina) *ad libitum*. Seven rabbits were characterized as severely hypertensive using criterion previously described (2, 4). These were selected from 27 rabbits, 30–40 days after surgical placement of a silver constricting clip (internal diameter of 0.7 mm) around the left renal artery. These rabbits had achieved blood pressure greater than 105 mm Hg as determined indirectly using a modified Grant–Rothchild capsule (6), and

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exhibited resting PRAs which were higher than normal values measured by radioimmunoassay after Haber *et al.* (7) as previously described (3). An additional 8 normotensive rabbits were studied as controls. All rabbits were anesthetized using a combination of phenobarbital, 125 mg/kg, followed 30 min later by pentobarbital, 10 mg/kg, and supplemented when necessary with additional pentobarbital via a catheter in the marginal vein of the ear. After tracheotomy, the femoral artery was cannulated using heparanized PE-90 tubing for continuous monitoring of arterial pressure with a Grass model 7D polygraph (Grass Instrument Co., Quincy, Mass.). The femoral vein was cannulated with PE-50 tubing to facilitate a constant maintenance infusion of isotonic saline at 100 μl/min. A laparotomy was performed to allow suprarenal placement of an adjustable aortic occluding loop such that renal perfusion pressure could be reduced in a stepwise fashion. This was passed exteriorly through a small incision in the left flank. A noncannulating electromagnetic flow probe (4 mm, Carolina Medical Electronics) was placed around the right renal artery as well as a small occluding loop distal to the probe used to produce a mechanical zero flow condition. These were passed to the exterior through a small incision in the right flank. The incision was closed and the rabbit suspended in an upright position to stabilize for 45 min. A series of pressure–flow curves were run by sequentially reducing the renal perfusion pressure by closing the aortic ligature.

To examine the contribution of the renin–angiotensin system to RBF, after a 30-min stabilizing period an intravenous dose of the competitive angiotensin antagonist [Sar^1-Ile^8]-AII was administered at a rate of 1 μg/min. After 15 min, the efficacy of the blocker was tested with a bolus injection of 1 μg of angiotensin II. Pressure–flow curves were repeated.

Results are presented in a fashion by which RBF has been normalized so that changes are given as a percent of the blood flow at a normalized initial BP. However, the actual blood flow rates are also provided. The pressure–flow relationships were derived by summing points within 10 mm Hg intervals from at least four repetitive curves run at least 10 min apart within each animal, after the method of Arendshorst *et al.* (8). Therefore, as a result of data accumulation, normalized blood pressure values presented on the figures appear slightly different from the direct initial resting values reported below. Values are reported as means ± one standard error. Analysis of statistical significance was performed using Student's t test for paired or unpaired variates, with $P < 0.05$ indicating a significant difference.

Results. Rabbits which were severely hypertensive 30–40 days after clipping of the left renal artery were characterized by higher PRA (30.1 ± 4 (SE) vs 11.5 ± 3 ng AI generated/ml/hr, $P < 0.001$) and were found to have significantly higher mean arterial blood pressures than did normotensive rabbits when measured either directly (96 ± 3 mm Hg vs 79 ± 3, $P < 0.001$) or by indirect means (115 ± 3 vs 80 ± 1 mm Hg).

When renal blood flow was compared in untouched kidneys of normotensive rabbits and not-clipped contralateral kidneys of severe renal hypertensive rabbits, the absolute flow rate was greater in the hypertensive rabbits (26.6 ml/min ± 3.2 vs 17.5 ± 2.7, $P < 0.025$). Thirty to forty days after renal artery clipping, the contralateral unclipped kidneys of hypertensive rabbits were hypertrophied such that they were larger than their matched clipped kidneys (12.4 g ± 1.7 vs 6.8 ± 0.8, $P < 0.002$) as well as the untouched kidneys of the normotensive rabbits (7.8 ± 0.5, $P < 0.005$). Thus, when renal blood flow was corrected by kidney weight, there was no difference between the contralateral kidneys of hypertensive and untouched kidneys of normotensive rabbits (2.4 ± 1.2 ml/min/g kw vs 2.4 ± 1.2 ml/min/g kw). However, total renal mass as a function of body weight was not different between hypertensive and normotensive rabbits (6.72 ± 0.29 g/kg bw vs 7.07 ± 0.68 g/kg bw). Figure 1 represents the renal pressure–flow relationships corrected for kidney weight in both normotensive and hypertensive rabbits. Because of variability in the renal pressure–flow relationship found between

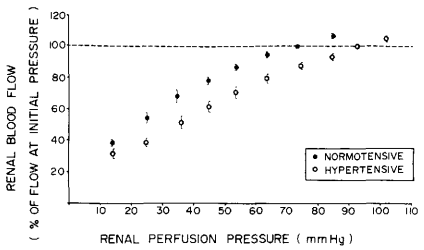

FIG. 1. Acute renal perfusion pressure–blood flow relationship in normotensive and in severe renal hypertensive rabbits. The 100% initial blood flow represents 2.4 ± 1.2 ml/min/g kidney wt.

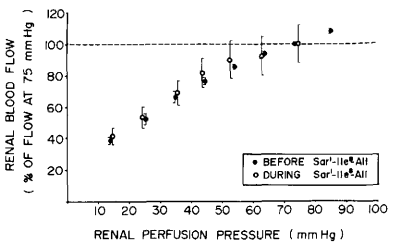

FIG. 2. Acute renal perfusion pressure–blood flow relationship in kidneys of normotensive rabbits before and during angiotensin blockade using [Sar¹-Ile⁸]-angiotensin II. The 100% initial blood flow represents 2.4 ± 1.2 ml/min/g kidney wt.

individual rabbits, flow has been normalized to a percentage of the RBF found at the initial or basal arterial pressure for both normotensive and hypertensive groups of rabbits. Importantly, this means that 100% of RBF in both groups was equal to 2.4 ± 1.2 ml/min/g kw. As renal perfusion pressure was sequentially reduced, RBF decreased proportionally in both groups, so the pressure–flow relationships are parallel. In the contralateral kidney of hypertensive rabbits, the curve is shifted to the right such that it requires a 27% greater perfusion pressure to achieve RBF which is similar to that found in the normotensive rabbits. When renal perfusion pressure of hypertensive rabbits was acutely reduced to normotensive levels, RBF decreased 13% ($P < 0.025$).

The contribution of the renin–angiotensin system to the renal pressure–flow relationship was studied by administration of the competitive angiotensin antagonist, [Sar¹-Ile⁸]-AII. When this blocker was administered to normotensive rabbits, there were no significant changes in either the basal BP, RBF, or RVR. When pressure–flow curves were run, as shown in Fig. 2, the results before and after blockade were not different and are superimposable. However, when the angiotensin antagonist was administered to severe hypertensive rabbits (Fig. 3), the BP dropped significantly ($P < 0.005$) to 60 mm Hg ± 7, a value which is not different from BP in normotensive rabbits after administration of the blockade. Renal vascular resistance decreased some 20% (4.0 ± 0.7 to 3.2 ± 1.4 PRU, $P < 0.025$) while RBF was maintained at a level similar to that found in normotensive rabbits. Therefore, the pressure–flow curve was shifted to a position similar to that obtained in normotensive rabbits both before and during angiotensin antagonism. The efficacy of the blocker was tested by administering a bolus injection of 1 μg of angiotensin II, and the [Sar¹-Ile⁸]-AII was found to abolish angiotensin-induced vasoconstriction.

Discussion. Our findings show that blood flow to contralateral not-clipped kidneys of severely hypertensive rabbits is maintained at a normal rate despite the elevation of renal perfusion pressure. The fact that absolute RBF was elevated can be wholly accounted for by the observed compensatory hypertrophy, characteristic of the contralateral kidney. It has been demonstrated (9, 10) that despite an exaggerated natriuretic and diuretic response of the contralateral kidney of two-kidney one-clip renal hypertensive rats to an intravenous saline

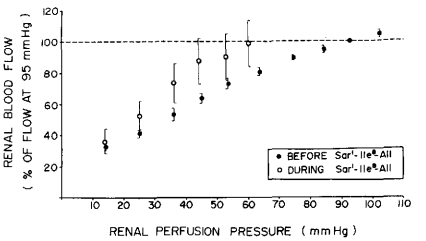

FIG. 3. Acute renal perfusion pressure–blood flow relationship in contralateral kidneys of severe renovascular hypertensive rabbits before and during angiotensin blockade using [Sar¹-Ile⁸]-angiotensin II. The 100% initial blood flow represents 2.4 ± 1.2 ml/min/g kidney wt.

load, glomerular filtration rate, and total excretion from both kidneys remains similar to that seen in normotensive rats. Observed clearance of PAH in these rats suggests that the absolute increase in RBF we observe in the hypertrophied contralateral kidney may be offset by an absolute decrease in RBF in the clipped kidney, such that the contralateral kidney adapts to maintain normal blood flow in an attempt to normalize overall renal function (10). Our values for RBF in the rabbit are 25% less than those reported by Ott and Vari (11) using an electromagnetic flow probe in rabbits with occluded carotid arteries. Likewise, our values are lower than those of Forster and Maes (12) found in renal denervated conscious rabbits. These differences may be due to the specific colony, the state of anesthesia, or the experimental protocol involved. Our values are similar to those previously reported from this laboratory in unanesthetized rabbits (3).

Severe renovascular hypertensive rabbits have been characterized by elevated plasma renin activity (PRA) (2, 4, 13) but the contralateral kidney has been found to be depleted of renin (14). Therefore, the elevated PRA is thought to derive from the clipped kidney (15) and be a major contributing factor to the elevated blood pressure in this form of hypertension (2, 5). It has been previously reported that only with severe hypertensive rabbits which are in negative sodium balance (5) or volume depletion (2, 4) does BP respond to angiotensin antagonism. We have shown that the administration of the angiotensin antagonist [Sar^1-Ile^8]-AII reduced the BP in severely hypertensive rabbits to a normotensive level, but RVR fell such that RBF was maintained. The same manipulation in normotensive rabbits had no effect. This is contrary to the response of RBF when renal perfusion pressure was reduced to normotensive levels prior to the administration of the blockers. In this case, RBF decreased significantly some 13%. These observations suggest that the increased RVR within the contralateral kidney which counteracts the high renal perfusion pressure and accounts for the shift in the renal pressure–flow relationship can be attributed to the vasoconstrictor properties of the elevated angiotensin in this form of hypertension.

We have previously shown (4) that the severe form of renovascular hypertension is associated with volume depletion possibly derived through a pressure related natriuresis and diuresis via the contralateral kidney as hypertension develops. Since we have shown that RBF is maintained in this model, it may be that the elevation of PRA is a function of the reported volume depletion, and its vasopressor action increases resistance thereby protecting the unclipped kidney from further losses of body fluid. So, while it is believed that stimulation of renin production due to renal artery stenosis may be an initiating stimulus for the development of this form of hypertension (16), it may ultimately also play a role protecting the contralateral kidney from the deleterious effects it has itself created.

Ploth and colleagues (17) have reported failure of the contralateral kidneys of renal hypertensive rats to autoregulate renal blood flow when compared with normotensive rats. We were not able to demonstrate typical autoregulation of renal blood flow in any of the rabbits studied, despite the presence of blood pressures and renal blood flows comparable to results reported in chronically studied conscious normal and hypertensive rabbits (3).

Forster and Maes (12) demonstrated autoregulation of renal blood flow in conscious renal denervated rabbits between 75 and 110 mm Hg (obtained by carotid artery occlusion). Ott and Vari (11) have reported autoregulation above 75 mm Hg in kidneys of anesthetized rabbits which have been made acutely hypertensive with carotid occlusion. Likewise, Eide and colleagues (18) report autoregulation in denervated normal rabbits whose BP was greater than 110 mm Hg and whose RBF was five times what we have observed or what has been reported in conscious rabbits (3). Notably, all these studies (11, 12, 18) show autoregulation of blood flow only above 75 mm Hg, approximately the normal arterial pressure of rabbits, a characteristic not present in other animal models (8). When we performed carotid artery occlusion and vagotomy on our

rabbits (unpublished observations), the arterial pressure was increased to hypertensive levels, but this was accompanied by significant attenuation of RBF, especially above 75 mm Hg. Such findings were somewhat similar to those of Forster and Maes (12) and Ott and Vari (11), but these acute conditions altered the basal pressure–flow relationships, obscuring our basic observation and therefore have not been included.

The rabbit is a difficult model with which to perform acute studies involving renal function. However, because it has previously proved an excellent model for describing the pathophysiology of severe renovascular hypertension (2–5), the inability to demonstrate acute renal autoregulation of blood flow should not detract from the essential observation that severe hypertensive rabbits chronically adapt RBF to apparently normal levels, despite the imposing and deleterious insult of extremely high renal perfusion pressure.

1. Möhring, J., Möhring, B., Näumann, H. J., Philippi, A., Homsy, E., Orth, H., Dauda, G., Kazda, S., and Gross, F., Amer. J. Physiol. **228**, 1847 (1975).
2. Romero, J. C., Holmes, D. R., and Strong, C. G., Circ. Res. **40**, (Suppl. 1), 17 (1977).
3. Romero, J. C., and Strong, C. G., Circ. Res. **40**, 35 (1977).
4. Schryver, S., Beierwaltes, W. H., Britton, S. L., Brown, R. D., and Romero, J. C., *Proc. 3rd Pan Am. Symp. Hyper.*, in press.
5. Lohmeier, T. E., and Davis, J. O., Amer. J. Physiol. **230**, 311 (1976).
6. Wilson, D. M., Romero, J. C., Strong, C. G., Lee, K. E., and Schryver, S., J. Lab. Clin. Med. **86**, 1032 (1975).
7. Haber, E., Koerner, T., Page, L. B., Kliman, B., and Purnode, A., J. Clin. Endocrinol. Metabol. **29**, 1349 (1969).
8. Arendshorst, W. J., Finn, W. F., and Gottschalk, C. W., Amer. J. Physiol. **228**, 127 (1975).
9. Kramer, P., and Ochwadt, B., Pflügers Arch. **332**, 332 (1972).
10. Peters, G., Brunner, H., and Gross, F., Nephron **1**, 295 (1964).
11. Ott, C. E., and Vari, R. C., Amer. J. Physiol. **237**, F479 (1979).
12. Forster, R. P., and Maes, J. P., Amer. J. Physiol. **150**, 534 (1947).
13. Romero, J. C., and Strong, C. G., Pract. Cardiol. **4**, 39 (1978).
14. Brunner, H., Desaulles, P. A., Regoli, D., and Gross, F., Amer. J. Physiol. **202**, 795 (1962).
15. Leenen, F. H. H., and deJong, W., Circ. Res. **36** (Suppl. 1), 179 (1975).
16. Freeman, R. H., Davis, J. O., Watkins, B. E., Stephans, G. A., and DeForrest, J. M., Amer. J. Physiol. **236**, 21 (1979).
17. Ploth, D. W., Rudulph, J., Roy, R., and Navar, L. G., Proc. Amer. Soc. Neph. **11**, 68A (1978).
18. Eide, I., Löyning, E., Aars, H., and Akre, S., Scand. J. Clin. Lab. Invest. **31**, 123 (1973).

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