GENETIC INFLUENCE ON THE DEVELOPMENT OF RENAL HYPERTENSION IN PARABIOTIC RATS

Evidence for a Humoral Factor*

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The relative roles of renal pressor and antihypertensive factors in the pathogenesis of experimental renal hypertension remain a central, and disputed, issue.

In our laboratory, we have evolved two strains of rats with opposite genetic predispositions to different forms of experimental hypertension including those produced by salt and unilateral renal artery constriction (1–4). But when a rat from each of the two strains was united in parabiosis, it was found that the reaction pattern to salt hypertension could be changed, i.e., the normally resistant animal not only developed significant hypertension but did so prior to its susceptible partner (5). This was interpreted to indicate that the genetic factors might act via a humoral agent, transmittable between rats from the two strains. The studies did not settle whether such factors were of a pressor or anti-pressor nature.

In the current study, we have explored the effects of a variety of renal manipulations in single and parabiotic animals. Our results suggest that, although intact renal tissue has an antihypertensive action, the loss of this function is not alone sufficient to explain renal hypertension; a pressor agent must be involved. Since only animals from the sensitive strain were able to induce experimental hypertension in their intact parabiotic partners, we have speculated that, in animals from the sensitive strain there are *two* pressor principles: One agent is present in animals from this strain as well as in the one resistant to hypertension and is not transmitted through the parabiosis junction; a second agent is peculiar to the strain predisposed to hypertension and will traverse the junction.

Material and Methods

The rats belonged to two strains developed in this laboratory, called R and S because of their resistance or sensitivity, respectively, to experimental hypertension. Detailed reports on

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breeding, feeding, care, and blood pressure measurements have been published (6–8) so that only details pertinent to the present studies will be included here. Standard Purina rat chow with a special low sodium content (0.4% NaCl dry weight) and tap water were used in all experiments. Statistical analysis was by standard techniques; some of the computations were performed on a computer by a program of analysis of variance.[1] P values $\leqq 0.05$ are considered significant.

Experiment 1

Uninephrectomy, Single Rats.—66 rats (11 males and 18 females from the R strain, 30 males and 7 females from the S strain) were used. 1 wk after weaning uninephrectomy was performed through incision over the lumbar region under ether anesthesia in 47 rats (27 S strain, 20 R strain) weighing 90–100 g. In 19 rats (10 S strain, 9 R strain), the lumbar incision was made but the kidney was not manipulated (sham-uninephrectomy).

After the operation, blood pressures and weights were measured every 2–4 wk for 14 wk.

Experiment 2

Unilateral Renal Artery Constriction, Single Rats. —This was done by the technique of Byrom and Wilson (9). This experiment was a repetition of one reported previously (3) and was done to make certain that no significant change in response had occurred in the R strain of rats in the intervening period. No sham-operated rats were included for controls but those from Experiment 1 may be so regarded.

12 R rats (7 males, 5 females) and 12 S rats (2 males, 10 females) were used.

5 wk after weaning, an annealed silver ribbon clip was applied to constrict the left renal artery, leaving the right kidney untouched. After the operation, blood pressures and weights were measured once a week for 12 wk.

Experiment 3

Unilateral Renal Artery Constriction with Contralateral Nephrectomy, Single Rats (Goldblatt Procedure).—38 rats (18 male R strain, 11 male and 9 female S strain) were used.

The operation was performed in two stages: In 21 male rats (10 R strain, 11 S strain), the right kidney was removed 1 wk after weaning as in Experiment 1. 4 wk after the first operation, a silver clip was applied to the left renal artery by the method described in Experiment 2. In 17 control rats (8 male R strain, 9 female S strain), the incision was made 1 wk after weaning but the right kidney was not manipulated. 4 wk after this operation, the left renal artery was exposed but the clip was not applied (sham-operation).

After the second operation, blood pressures were measured once a week. In the operated rats, death of S strain rats during the next 3 wk left a final total of 13 rats on which this analysis was based at the end of 4 wk. After 4 wk the mortality was so high that meaningful comparisons were impossible.

Experiment 4

Unilateral Renal Artery Constriction and Contralateral Nephrectomy of One Partner in Parabiosis. —The operation was performed in two stages: Weanling rats were united in parabiosis by the method of Bunster and Meyer (10); this union includes skin and subcutaneous tissue together with the scapulae for fixation. The circulatory connection is by both capillary and venous anastomosis in the skin and subcutaneous tissue. The rats were arranged so that the partner to have the Goldblatt procedure had its right side towards the midline of the

[1] We thank Keith Thompson for help with this program.

pair, and the right kidney was removed from this animal at the time of parabiosis. 70 pairs in the following combinations resulted from this first operation:

R*-R	17 pairs	S*-R	16 pairs
R*-S	17 pairs	S*-S	20 pairs

(*Indicates operated animal)

4 wk after the first operation, a silver clip was applied to the contralateral (left) renal artery of the uninephrectomized partner by the method described in Experiment 2. Blood pressures and weights were measured once a week for 12 wk thereafter.

OBSERVATIONS

Experiment 1

Effects of Uninephrectomy on Single R and S Rats on Low Salt Diets. Uninephrectomized S rats developed a mild hypertension compared with the sham-operated control group, reaching a plateau at approximately 4 wk. R rats showed no difference between sham-operated and uninephrectomized groups (Table I, Fig. 1).

There was no mortality in this group.

Experiment 2

Effect of Unilateral Renal Artery Constriction in Single R and S Animals on Low Salt Diets.—The results were similar to those reported previously (3) except that in the first study males had higher blood pressures than females, a difference that was not significant in the present study (Table II, Fig. 2).

All S rats developed hypertension from which four died during the 12 wk of observation. R rats were far less vulnerable: While most developed mild hypertension, some remained within normal limits; only one R rat died, during the 6th wk, with its highest recorded blood pressure being 148 mm Hg. Within each strain, there was no significant difference in mean blood pressure after the 4th wk; in the S group this was partly due to the death of four rats with very high blood pressures during the 6th and 7th wk. Average blood pressures in both strains were significantly higher than those seen after uninephrectomy.

Experiment 3

Effects of Unilateral Renal Artery Constriction and Removal of Contralateral Kidney (Goldblatt Procedure) in Single R and S Animals on Low Sodium Diet.—The results differed from those obtained with unilateral renal artery constriction (Experiment 2) in that here the R as well as the S rats rapidly developed severe experimental hypertension of approximately equal degree and gravity (Tables III A and B, Fig. 3). The disease was of much greater severity than that in Experiment 2 as shown by the mortality which was so high that meaningful comparisons between the strains could not be made beyond the 4th week and all animals were dead after 10 weeks.

There was no hypertension or death in the control groups; the rats were sacrificed after 14 weeks.

Experiment 4

Effect on Both Animals of Goldblatt Procedure on Only One Partner in Parabiosis. Low Sodium Diet.—70 pairs of rats were united in parabiosis. After the

TABLE I

Experiment 1. Effects of Uninephrectomy in Single R and S Rats on Low Salt Diet

	R-sham (control)			R-operated			S-sham (control)			S-operated		
	Sex	4 wk	8 wk	Sex	4 wk	8 wk	Sex	4 wk	8 wk	Sex	4 wk	8 wk
Systolic blood pressure (*mm* Hg)	Male	120	128	Male	126	120	Male	136	138	Male	140	142
	"	136	124	"	130	118	"	128	136	"	142	140
	"	130	126	"	130	130	"	132	130	"	158	150
	"	114	118	"	128	124	"	130	126	"	136	140
	Female	126	126	"	136	120	"	128	124	"	140	152
	"	140	134	"	122	118	"	130	138	"	168	156
	"	136	126	Female	122	118	"	138	120	"	154	150
	"	126	130	"	126	128	"	134	144	"	140	158
	"	124	128	"	126	128	"	144	140	"	136	132
				"	122	120	"	130	140	"	148	154
				"	120	124				"	126	138
				"	140	132				"	140	144
				"	126	120				"	158	156
				"	130	122				"	134	146
				"	118	120				"	148	160
				"	136	130				"	140	142
				"	120	118				"	140	150
				"	128	120				"	144	140
				"	130	134				"	154	168
				"	130	128				"	162	160
										Female	138	140
										"	148	164
										"	154	156
										"	144	138
										"	150	158
										"	146	150
										"	144	140
No. of rats	9			20			10			27		
Mean blood pressure....		128	127		127	124		133	134		146	149
SE.....		2.8	1.5		1.3	1.2		1.6	2.5		1.8	1.8
Median blood pressure....		126	126		127	121		131	137		144	150

Blood pressures were taken through the 12th wk but the results shown are those obtained 4 and 8 wk after operation, at which time the values had reached a plateau. A comparison by *t* test at 8 wk indicates:

R sham = R operated

R operated < S sham ($P < 0.025$)

S sham < S operated ($P < 0.001$)

Sexes were analyzed separately but since no difference in blood pressure could be demonstrated, the results were pooled for this analysis.

completed Goldblatt procedure, immediate postoperative mortality plus death during the next few weeks left a total of 48 pairs alive and in good health at 8 wk. (Using an asterick [*] to indicate the partner with the Goldblatt procedure, the distribution was as follows: 11 R*-R, 14 R*-S, 12 S*-R, and 11 S*-S). At 12 wk there were 43 pairs alive (9 R*-R, 14 R*-S, 12 S*-R, and 8 S*-S). The detailed blood pressure data at 8 and 12 wk are summarized in Table IV. In

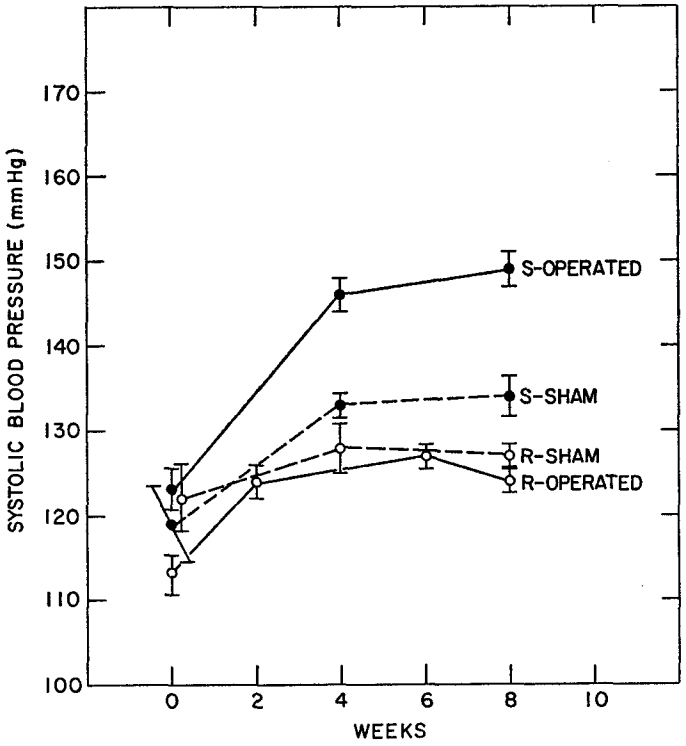

FIG. 1. Effect of uninephrectomy in single R and S rats on low salt diet. (See legend for Table I).

Figs. 4–7, the mean biweekly blood pressure values through the 12th wk include the data on all pairs in good health at the time blood pressure was measured.

The most striking difference in response among R and S parabionts was that when the Goldblatt procedure was carried out on an S animal, hypertension developed in both the operated rat and its intact partner whereas the same procedure on an R rat caused hypertension in that animal but little or none in its partner. This did not appear to be related simply to the level of blood pressure in the operated S rat. For instance, by the 8th wk average blood pressure was at similar levels in the operated partners of the S*-R, R*-S, and R*-R combina-

tions (170, 169, 179 mm Hg, respectively, $P > 0.05$); yet hypertension, while well established in the intact partners of the operated S rats, was absent in the intact partners of the operated R animals.

The Goldblatt procedure produced a much less fulminant disease in the operated rat when it was in parabiosis with an intact rat from either strain than

TABLE II

Experiment 2. Effect of Unilateral Renal Artery Constriction in R and S Rats on Low Salt Diet

	S rats			R rats		
	Sex	4 wk	8 wk	Sex	4 wk	8 wk
Systolic blood pressure (*mm* Hg)	Female	230	①*	Male	178	174
	"	160	160	"	144	144
	Male	246	①	"	148	①
	"	170	244	"	136	146
	Female	176	188	"	138	146
	"	222	①	"	134	148
	"	160	170	"	130	126
	"	250	①	Female	140	136
	"	162	160	"	126	130
	"	160	174	"	140	140
	"	174	188	"	136	140
	"	168	180	"	134	136
No. of rats		12	8		12	11
Mean blood pressure		190	183		140	142
SE		10.4	9.5		3.8	3.8
Median blood pressure		172	177		137	140

* ①, dead.

Operation was performed on animals approximately 4 wk of age. Blood pressures were obtained at time of operation and thereafter once a week for 12 wk. No significant sex difference could be demonstrated. Blood pressures reached a plateau by the 4th wk after which there was no significant difference in mean blood pressure within each strain ($P > 0.05$); the difference between strains was significant ($P < 0.01$).

when applied to single animals. This was manifested by a slower evolution of hypertension and a lesser mortality. Thus it took 6–10 wk for the operated members in parabiosis to develop blood pressures of 180–190 mm Hg, levels reached by similarly operated single animals within the first 2–3 wk. And, while the single animals were all dead by the end of the 10th postoperative week, 40–80% of the original parabionts in each group were still alive at this time. It seems reasonable to suggest, therefore, that the intact rats somehow protected their operated partners. This protection was not consistently related to the

strain from which the intact animal derived, as shown by the following relationships among the average blood pressures of operated animals at both 8 and 12 wk: S* (S) > S* (R) ≥ R* (S) = R* (R) (strain of intact partner in parenthesis).

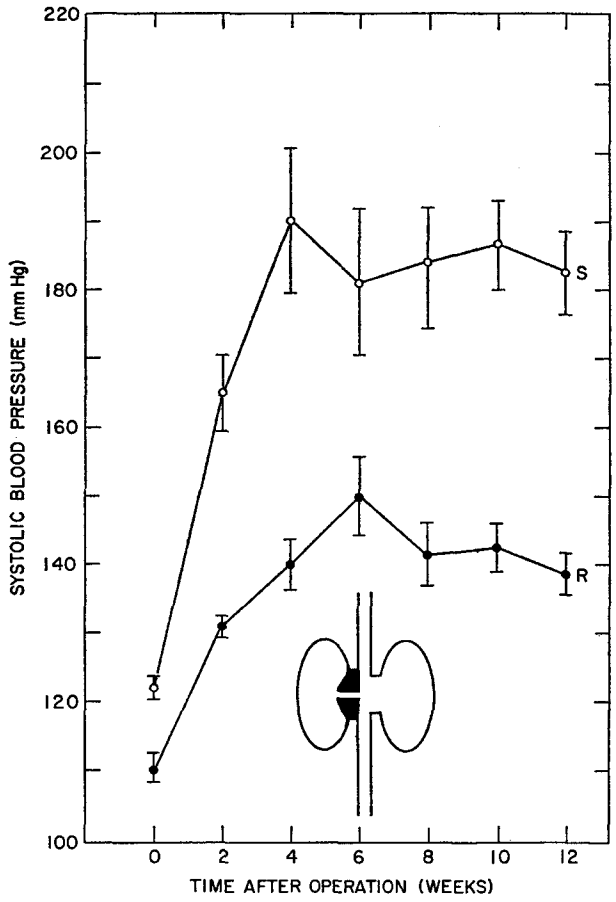

FIG. 2. Mean blood pressure in single R and S rats on low salt diet with unilateral renal artery constriction. At all times, including day of operation (0 wk), the S group had significantly higher mean blood pressure than the R group ($P < 0.01$). (See legend for Table II.)

DISCUSSION

In this study, we explored the effects of different renal manipulations on two strains of rats with opposite constitutional predispositions to experimental hypertension. The following observations were made: (1) Uninephrectomy produced mild hypertension in rats on low NaCl diets from the sensitive (S)

TABLE III

Experiment 3. A. Effect of Unilateral Renal Artery Constriction with Contralateral Nephrectomy in R and S Rats on Low Salt Diet

	S-rats				R-rats			
Week	0	2	3	4	0	2	3	4
Systolic blood pressure (*mm* Hg)	134	180	204	—*	126	190	192	188
	136	168	210	200	124	170	178	182
	142	—			126	166	176	190
	128	186	200	204	128	188	200	210
	130	—			124	158	186	—
	130	182	170	176	122	162	170	180
	138	218	196	186	128	186	180	198
	140	180	170	174	130	156	178	190
	126	—			126	—		
	132	—			128	162	188	180
	140	192	216	—				
No. of rats	11	7	7	5	10	9	9	8
Mean blood pressure...	134	187	195	188	126	171	183	190
SE	1.5	5.9	6.9	6.1	0.8	4.5	3.1	3.6

B. Effect of Unilateral Renal Artery Constriction with Contralateral Nephrectomy in Control R and S Rats on Low Salt Diet (Sham-Operated)

	S-rats				R-rats			
Week	0	2	3	4	0	2	3	4
Systolic blood pressure (*mm* Hg)	126	128	126	132	130	128	130	126
	120	128	120	128	122	126	124	126
	112	128	120	124	124	126	120	124
	136	140	136	126	126	128	120	114
	126	120	134	136	120	132	120	116
	128	134	130	140	122	122	120	122
	128	120	132	138	132	128	138	120
	126	136	130	122	128	124	126	130
	128	128	128	126				
No. of rats	9	9	9	9	8	8	8	8
Mean blood pressure...	126	129	128	130	126	127	125	122
SE	2.2	2.2	1.9	2.2	1.5	1.1	2.3	1.9

* —, died.

The column "0 week" presents results obtained after 4 wk of uninephrectomy and may be compared with those presented in Table I. S rats developed Goldblatt hypertension at a somewhat faster rate than R animals, but after 4 wk, the systolic blood pressures were equivalent ($P > 0.05$).

Blood pressures were taken through the 10th wk although a plateau was reached by the 4th wk. The mortality increased rapidly after the 4th wk and, by the end of the 10th wk, all rats in both strains were dead. If the highest blood pressure on record for each rat is used for comparison, the average for the R group is 188 ± 5.0 mm Hg and for the S group, 195 ± 7.5 mm Hg. The difference is not statistically significant ($P > 0.05$).

The control animals all remained normotensive and there were no deaths in this group.

but not in members from the resistant (R) strains; (2) unilateral renal artery constriction (only) produced severe hypertension in members of the S strain on low NaCl diet but no, or only mild, hypertension in rats from the R strain genetically refractory to it; (3) unilateral renal artery constriction and contralateral nephrectomy (Goldblatt procedure) resulted in fulminating hypertension of approximately equal severity in both strains; (4) the Goldblatt procedure

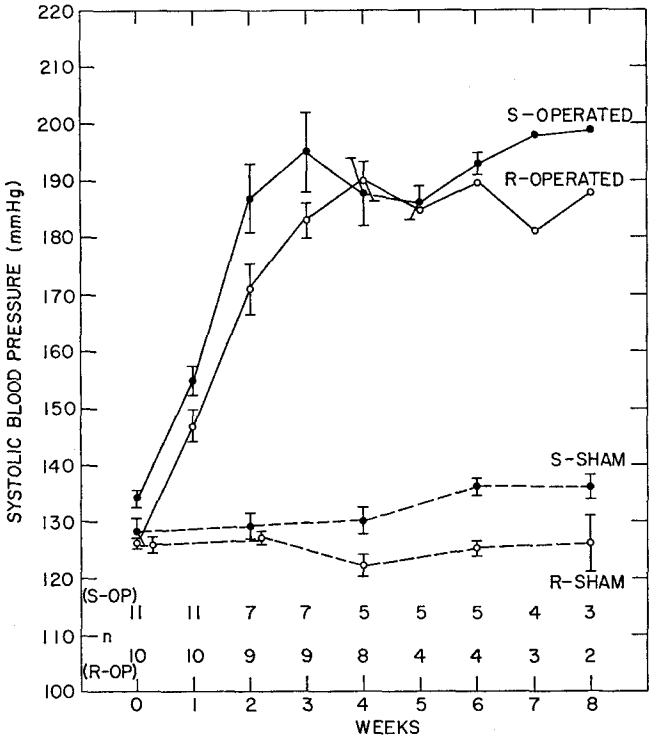

FIG. 3. Effect of unilateral renal artery constriction with contralateral nephrectomy in R and S rats on low salt. n, number of rats. (See legend for Table III.)

carried out on only one member of a parabiotic pair (*a*) induced hypertension in the operated rat but significant hypertension developed in the intact partner only when the operated animal belonged to the S strain and (*b*), compared with single similarly operated animals, produced a more benign hypertension in the operated rat, suggesting that the intact partner somehow ameliorated the hypertensive process.

The uninephrectomy study demonstrated the protective value of intact renal tissue and indicated genetically determined difference in the ability of the kidneys to protect against the development of hypertension. This was shown most

graphically by the different response of R rats to unilateral renal artery compression in the presence or absence, respectively, of the intact contralateral kidney. In the presence of an intact kidney, the result was mild hypertension, at most, whereas in its absence severe and fatal hypertension rapidly developed.

TABLE IV

*Experiment 4. Effect of Blood Pressure of Goldblatt Procedure on Only One Partner in Parabiosis**

Pair	S*-S				R*-S				S*-R				R*-R			
Partner	S*		S		R*		S		S*		R		R*		R	
Week	8	12	8	12	8	12	8	12	8	12	8	12	8	12	8	12
Systolic blood pressure (*mm* Hg)	174	178	150	150	164	160	144	142	148	166	150	150	170	186	130	140
	200	232	174	170	150	164	148	142	172	188	160	168	166	180	134	132
	164	178	156	166	202	190	136	154	192	208	184	186	224	186	144	132
	176	194	184	176	176	190	132	136	170	192	158	162	186	178	132	130
	176	222	160	196	174	186	138	130	144	168	138	140	176	178	130	134
	208	206	176	190	170	202	130	144	148	174	146	146	170	180	144	142
	206	220	178	186	142	154	124	134	200	218	152	166	162	168	130	126
	192	210	184	188	148	156	132	140	146	186	140	154	180	—	138	—
	216	—‡	190	—	178	196	150	154	184	186	150	150	156	182	134	140
	194	—	168	—	170	202	130	156	188	200	144	184	180	—	128	—
	186	—	154	—	196	186	160	166	168	186	148	150	194	190	144	156
					176	178	142	140	184	222	170	188				
					162	180	134	144								
					156	176	128	128								
No. of rats	11	8	11	8	14	14	14	14	12	12	12	12	11	9	11	9
Mean blood pressure	190	205.0	170	177.8	169	180	138	143.4	170	191.2	153	162.0	179	180.8	135	136.9
SE	5.0	7.1	4.1	5.4	4.6	4.3	2.7	2.9	5.7	5.2	3.8	4.8	5.6	1.9	1.9	3.0
Median blood pressure	192	208	174	181	170	183	135	142	171	187	150	158	176	180	134	134

* S* and R* indicate animals with Goldblatt procedure.

‡ —, died.

The most significant observation here is that the Goldblatt procedure in an S rat caused hypertension in both the operated rat and its intact partner (see S*-S and S*-R) whereas the same procedure on an R rat caused hypertension in that animal but little or none in its partner (see R*-S and R*-R). The average blood pressure of the operated rats in the S*-S pairs was higher than that of any other group ($P < 0.04$–0.001) at all times. At 12 wk, the average blood pressure of the operated rats in S*-R was higher than that of the operated rats in R*-S ($P < 0.036$); the average blood pressure of the operated rats in the R*-S and the R*-R groups were equal ($P > 0.05$). Therefore, the blood pressure of the intact partner was not directly related to the blood pressure of its operated partner.

Indeed, lacking the contralateral kidney, the R animals developed hypertension that was indistinguishable from that in S rats similarly treated. The results do not indicate whether such protection is mediated by release of an anti-pressor agent, by destruction of an extrarenal pressor factor, or whether it just represents one facet of kidney function in general.

The uninephrectomy study, taken by itself, would be compatible with the

original thesis of Grollman that intact renal tissue is essential for maintenance of the normotensive state and that renal hypertension is due, not to a pressor factor, but to absence of this normotensive activity (11). This explanation would not suffice, however, for the fact that unilateral renal artery constriction

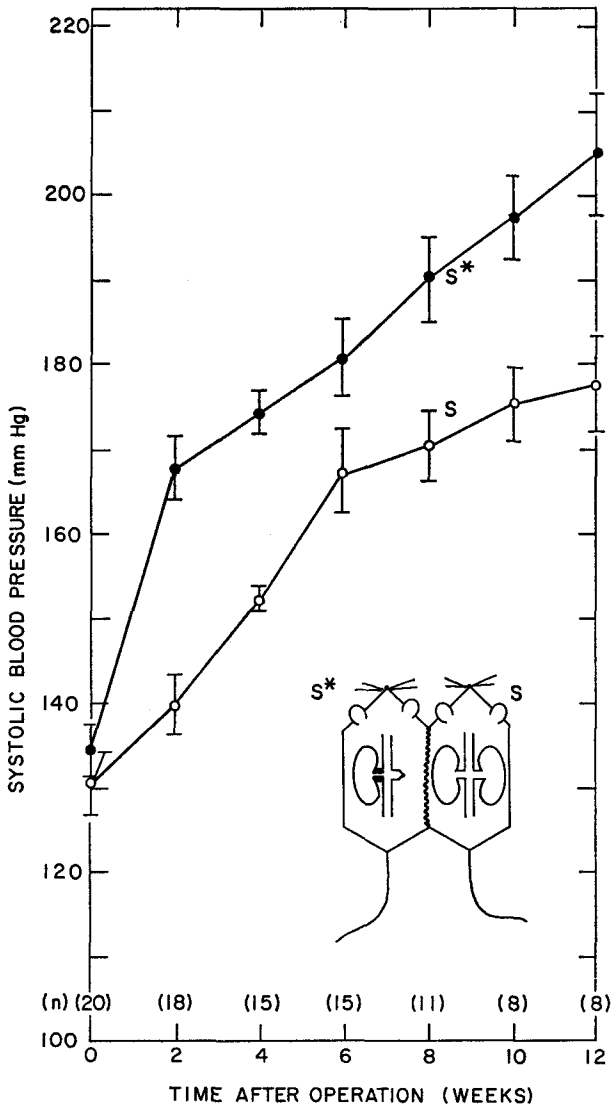

FIG. 4. Blood pressure response of S*-S parabionts after Goldblatt procedure in only one rat (S*).

produced much higher blood pressures than unilateral nephrectomy. Such results cannot be explained merely by absence of normotensive activity. In general terms, renal artery constriction must cause release of a pressor agent, most likely produced in the kidney itself or as the result of a tropic agent re-

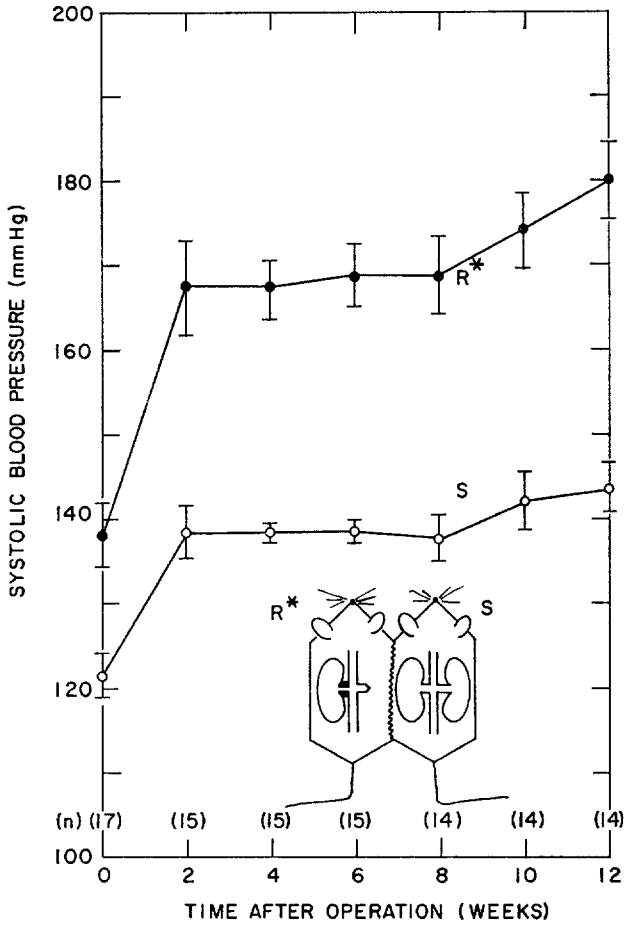

FIG. 5. Blood pressure response of R*-S parabionts after Goldblatt procedure in only one rat (R*).

leased from it. As a first approximation, it would seem appropriate to assume that the renin-angiotensin system was involved. It is known that reduction of functional renal mass leads to adaptive processes; if these adaptations differed either quantitatively or qualitatively in R and S rats, this might lead to hypertension in the latter. Since renal artery constriction also involves a reduction in

functional renal mass, the same adaptive processes would be acting as they do in uninephrectomy. In addition, however, there is the pressor signal released because of the renal artery constriction, presumably due to reduced arterial pressure in the "clipped" kidney. This signal would be expected to cause hyperten-

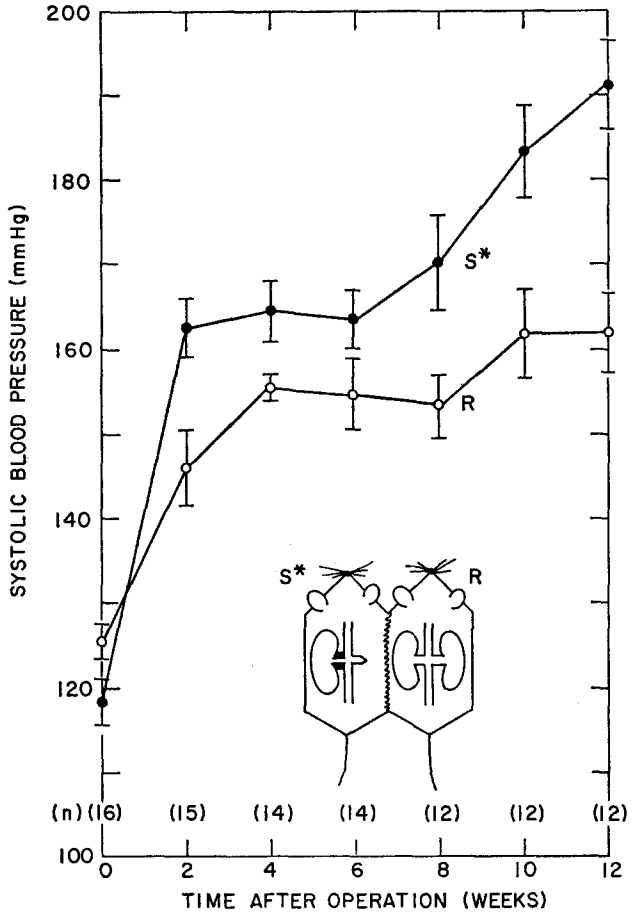

FIG. 6. Blood pressure response of S*-R parabionts after Goldblatt procedure in only one rat (S*).

sion in the affected animals. However, if in R animals the intact kidney can partly or wholly neutralize the signal, this might explain why the blood pressure increases only moderately or not at all. In S animals, by contrast, if the intact kidney has but a limited capacity to neutralize the pressor action, severe hypertension results. In the absence of this intact kidney, as in the classical Goldblatt

procedure used here in Experiment 3, it would be expected that R and S animals would be equally helpless to prevent hypertension. This was found to be the case. Taken together, the uninephrectomy and unilateral renal artery constriction studies suggest that at least two factors are at work here: one that "pro-

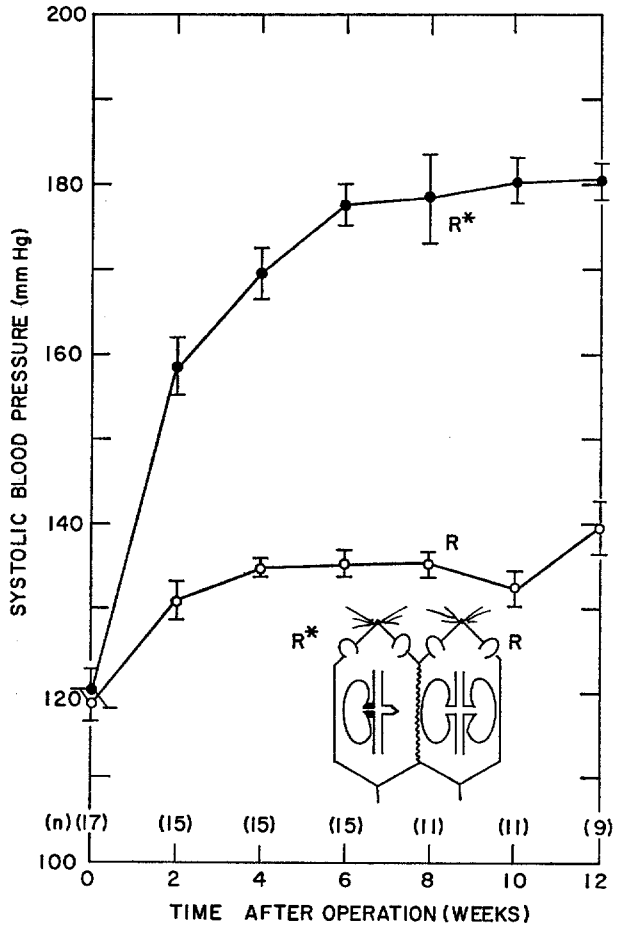

FIG. 7. Blood pressure response of R*-R parabionts after Goldblatt procedure in only one rat (R*).

tects" against hypertension and another that "promotes" it. This general formulation approximates that of Grollman's more recent proposal (12).

When these speculations are applied to the results of the parabiosis study, it becomes apparent that the foregoing explanation will not satisfactorily explain all of the observed phenomena. If the pressor signal were identical in the two

strains, the kidneys of the intact R should neutralize that fraction of the pressor material which crosses the parabiosis juncture, without development of hypertension, whereas the intact S should fail to neutralize the pressor material and *would* develop hypertension. But this was not the case: Hypertension was induced in either an R or S intact rat only if the operated partner was from the S strain. Therefore, there must be a difference in the pressor signal from the two strains. The simplest explanation is that this is a *quantitative* difference, with the S strain releasing significantly greater amounts than the R. As judged by the similarity in response to the Goldblatt procedure of single animals from both strains, a quantitative difference might be thought unlikely. It is possible, however, that the fulminating course in all such single animals obscured subtle quantitative differences that do exist between the strains.

Another possibility, more intriguing to us, is that there are *qualitative* differences in the pressor signals of the two strains. We have already reviewed the evidence suggesting that, in single animals, the kidneys of the R strain have great capacity to inactivate (or suppress) the pressor signal. In the S*-R pair, the pressor signal is transferred to the intact R animal via the parabiosis junction, presumably in smaller amounts than that which a single R rat handles readily if it has one intact kidney with the other renal artery constricted. But in the S*-R parabiont, the R developed hypertension. This suggests that there may be a qualitative difference in the pressor signal(s) released by the two strains. That is to say, the S strain has a hypertensinogenic substance not shared by the R strain.

The findings permit the speculation that at least two pressor principles are present in S rats with hypertension induced by the Goldblatt procedure: one agent which is common to R and S animals and which is not transmittable in parabiosis, and a second agent which is specific for the S strain but which is transmitted through the parabiosis junction. We may further speculate that the transmittable agent is identical with the factor which produces salt hypertension in these animals and is associated with the salt-excreting mechanism.

SUMMARY

The effects of several renal manipulations including uninephrectomy, unilateral renal artery constriction, and a combination of these two (Goldblatt procedure) were studied in two strains of rats with opposite constitutional predispositions to experimental hypertension. The protective value of intact renal tissue to protect against hypertension was shown to be genetically determined.

The Goldblatt procedure carried out on only one member of a parabiotic pair induced hypertension in this operated rat but significant hypertension developed in the intact partner only when the operated animal belonged to the strain predisposed to hypertension.

It was speculated that there were qualitative differences in the pressor signals

of the two strains of rats. In the strain genetically predisposed to hypertension there are at least two pressor principles: (*a*) one which is common to both strains, not transmittable via the parabiosis junction and presumably related to the renin-angiotensin system; and (*b*) a second which is specific for the hypertension-prone strain and can be transmitted through the parabiosis junction. This transmittable agent is probably identical with the factor that produces salt hypertension and is associated with the salt-excreting mechanism.

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