Release of Pressor Substances from Renal Grafts Originating from Rats with Renal Hypertension

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Transplantation of normal kidneys into 18- to 24-hour nephrectomized rats caused a sharp and sustained pressor response. By contrast, transplantation of partially infarcted kidneys produced only a slight or no rise in arterial pressure. This decrease, or lack of pressor activity, in the renal vein effluent was observed within a few days after infarction before development of hypertension and persisted in all rats which became hypertensive, as well as in some which remained normotensive. Two factors limited the significance of these results: a period of renal ischemia during the grafting procedure and the method of producing renal hypertension. Indeed, there is no indication that hypertension elicited by partial renal infarction has the same pathogenic mechanism as that involved in hypertension caused by other renal manipulations. We now describe a method of transplantation without renal ischemia and extend the study of the release of pressor substances to kidneys originating from rats made hypertensive by the more accepted procedures of either renal artery or parenchyma constriction.

Methods

Sprague-Dawley rats were used; they were fed on Purina fox chow and given tap water as drinking fluid. The grafting procedure consisted of anastomosing the lower abdominal aorta and vena cava of a donor rat to the femoral blood vessels of a nephrectomized recipient without ever interrupting blood supply to the renal graft. Vascular anastomoses were done with polyethylene tubing; sizes were chosen to permit a large blood flow and easy connections.

Preparation of the Recipient

A rat weighing between 190 and 225 Gm., matched according to weight and sex with the donor rat, was bilaterally nephrectomized under ether anesthesia. Eighteen to 24 hours later, it was anesthetized with sodium Amytal (8 mg./100 Gm. of body weight), administered intraperitoneally, and tied to the top of a flat rectangular box 5 cm. high with its left back corner cut open to permit insertion of the board used for the donor animal; a small rod anchored on the box maintained the right hind leg of the rat in an extended position (fig. 1). Following dissection of the neck, the trachea, carotid artery, and jugular vein were cannulated. The catheter (no. 60) inserted into the carotid artery was connected to a small-bore mercury manometer for blood pressure recording; the one (no. 50) in the jugular vein was used for injection of test substances. Then the right inguinal area was dissected, the sciatic nerve cut, and the femoral artery and vein cannulated with nos. 50 and 100 tubing, respectively. All catheters inserted into blood vessels were filled with heparin solution (10 mg./cc.) and plugged at their distal ends with a mandrel until connections were established. The rat was heparinized by flushing 1 mg. of heparin into the femoral vein. Blood pressure was then recorded; when stabilized, the sensitivity of the rat was tested by injecting isoleucine 5 angiotensin II at the dose levels of 0.01, 0.02 and 0.04 units. Corresponding responses were fairly constant; they averaged 9, 11, and 15 mm. Hg respectively. Any rat showing an abnormally low pressor response to test doses of angiotensin was discarded.

Preparation of the Donor

The donor was anesthetized with sodium Amytal (9 mg./100 Gm. of body weight) and injected

*Graciously furnished by Dr. E. W. Young of the Upjohn Company.
subcutaneously with 3 mg. of heparin. It was fastened to a special board consisting of a piece of wood about 8 cm. wide and 23 cm. long, cut on a slant so that its height at both ends was 8 and 3 cm. respectively. Intestines and spleen were then removed to facilitate dissection and maintain blood pressure at near normal levels. The left kidney is preferred to the right as a graft because it has a longer pedicle and is not covered by the liver. Spermatic or ovarian vein and adrenal vein were tied, as well as all the visible branches coming out of the aorta and vena cava from below the origin of the right renal pedicle caudad to a distance of approximately 1 cm., with the exception of the left renal artery and vein. Then the ureter was cut and the kidney freed with a cautery from the surrounding fat tissue. The kidney was transposed toward the center of the abdominal cavity so that the side touching the abdominal wall was exposed; with the help of a thread, the vena cava and aorta were raised to permit tying of the two or three lumbar arteries and veins which originate from the dorsal surface. A clamp was placed on the aorta and vena cava close to and below the renal pedicle. These vessels were cannulated with nos. 100 and 200 polyethylene tubing, respectively. Rat and board were then inserted into the opening provided on the left back corner of the first board used for the recipient rat (fig. 1). While blood circulation was temporarily interrupted in femoral blood vessels of the recipient, cannulae inserted in these vessels were connected with those in aorta and vena cava of the donor; then the clamp located just below the origin of the left renal pedicle was released at the same time as both aorta and vena cava were ligated above this pedicle, therefore establishing perfusion of the kidney with blood from the nephrectomized recipient. It took about 15 minutes to prepare the donor rat. In some instances, transplantation was done following washing of the kidney with physiological saline; this was accompanied by a period of renal ischemia of about 10 minutes. As will be seen later, the results obtained with the two procedures were not significantly different, suggesting that the small amount of blood trapped in blood vessels and kidney of the donor rat had no effect per se on the blood pressure of the recipient.

The donor rats were either normal or hypertensive. Hypertension was produced in female rats weighing from 130 to 160 Gm. by constricting the renal artery with silver clips with an opening of 0.2 mm. Wrapping of the kidney was done in rats weighing 120 to 140 Gm. according to the method of Kempf and Page. Since only the left kidney was used as graft, unilateral constriction of the renal artery or encapsulation was performed on either side depending on which kidney, the manipulated or its contralateral, was to be transplanted. In some instances, these procedures were associated with contralateral nephrectomy. Hypertension was also produced in a small group of rats by partial renal infarction. Blood pressure was determined by sphygmomanometry. After perfusion, kidneys, heart, and mesentery of the donor were removed and prepared for histological examination.

Results
Effects of Normal Kidneys
Transplantation of normal kidneys without ischemia caused a pressor response consisting usually of an initial fast rise followed by a more gradual one to a plateau, which was maintained throughout the period of observation (fig. 2). Each rise in pressure amounted to about 10 mm. Hg. The first one lasted 3 to 6 minutes and the second 15 to 40 minutes. Base-line pressures prior to grafting were between 70 and 122 mm. Hg. For comparative purposes, we measured arbitrarily the height of the response 15 minutes after grafting. The mean value for normal kidneys was $13.4 \pm 1.61$ mm. Hg (fig. 3). Of 16 normal kidneys, 10 gave this typical response, 4 gave only a sharp rise followed by a plateau, and 2 an insignificant response. Under similar

Figure 1
Diagram of experimental arrangement for transplantation of a kidney without renal ischemia. The kidney of the donor rat is being perfused with blood from a recipient rat through cannulae inserted into femoral blood vessels and aorta and vena cava. Note the clamp on aorta and vena cava above the renal pedicle.
Pressor responses elicited by a normal kidney grafted without ischemia. Grafting (1); removal of the graft (2).

Pressor effects of clipped and untouched kidneys grafted on nephrectomized rats. Dots represent changes in blood pressure 15 minutes after grafting. Transverse lines with shaded area indicate mean and standard error of values obtained from grafts without ischemia.

Effects of Kidneys with the Renal Artery Constricted

Rats with Unilateral Constriction and Contralateral Nephrectomy

Values of arterial pressure of donor rats at the time of testing varied between 170 and 220 mm. Hg (average 206 mm. Hg). Duration of hypertension from the time blood pressure values had reached 150 mm. Hg varied between 4 and 84 days, or 18 and 101 days after clipping. Of 12 kidneys, 9 were transplanted without ischemia and 3 with ischemia. With the exception of 2 kidneys in which perfusion was apparently inadequate as shown by lack of venous return, the remaining 10 kidneys gave definite pressor response with both methods of transplantation (figs. 4 and 5). Increments in blood pressure after 15 minutes of perfusion averaged 18.4 ± 2.64 mm. Hg (fig. 3) in the group with no ischemia. This rise was not statistically different from that obtained with normal kidneys (0.11 < P < 0.10). In five of these animals, the clip was successfully removed during perfusion when blood pressure had reached a plateau; in two of them, blood pressure fell gradually toward its original level (fig. 4); and in the other three, there was no change.

Rats with Unilateral Constriction of the Renal Artery Without Contralateral Nephrectomy

Of 13 rats with unilateral clipping of the renal artery, 10 had pressures over 200 mm. Hg and 3, pressures below 150 mm. Hg. All kidneys gave a pressor response, but it was less in the three which originated from rats...
Figure 4
Pressor response elicited by a clipped kidney grafted without ischemia. Response to 0.02 and 0.04 units of angiotensin (1); grafting (2); removal of graft (3).

with the lowest blood pressure. Kidneys transplanted without ischemia gave rises in blood pressure averaging 21.5 ± 2.49 mm. Hg (fig. 3). This value is statistically different from 13.4 mm. Hg given by kidneys from normal rats (P < 0.01).

Clipped kidneys were atrophic but free of lesions, while the opposite kidneys showed definite lesions of nephrosclerosis. Weights of clipped kidneys and their untouched counterparts averaged, respectively, 346 and 459 mg. per 100 Gm. of body weight. The clip was removed without hemorrhage in six kidneys when the pressor response was maximum. This was associated with a fall in pressure in only one of them.

Effects of Kidneys Contralateral to Clipped Kidneys

Of 17 animals with unilateral clipping of the renal artery, 11 became hypertensive and 6 remained normotensive. The contralateral kidneys from hypertensive animals, when transplanted with and without ischemia between the twenty-sixth and sixty-fourth day after operation, caused no rise in pressure, but rather a decrease in most cases or no significant change (fig. 6). Blood pressure responses measured 15 minutes after grafting averaged -10.25 ± 3.86 mm. Hg (fig. 3).

Kidneys from rats which had remained normotensive gave a slight pressor response.

On histological examination, grafted kidneys originating from hypertensive rats showed lesions of nephrosclerosis. Weights of clipped and untouched kidneys averaged, respectively, 346 and 449 mg. per 100 Gm. of body weight, values almost identical with those reported above. In one experiment, blood supply to the kidney was interrupted at the time of the depressor effect; this was associated with partial return of the pressure to its original level.

Effects of Wrapped Kidneys

Rats with Unilateral Kidney Wrapping and Contralateral Nephrectomy

Transplantation of 11 wrapped kidneys was done between the ninth and ninety-ninth day after operation when arterial pressure had reached values between 170 and 210 mm. Hg (average 195 mm. Hg). Five kidneys transplanted without ischemia elicited either no change or a depressor response. Six kidneys gave an insignificant pressor response, or no change, in spite of a period of as much as 20-minute ischemia (fig. 7). Responses after 15 minutes of observation averaged -3.6 mm. Hg without and +4 mm. Hg with ischemia (fig. 8).

At autopsy, it was found that as a result of compensatory hypertrophy, part of the kidney had expanded outside the wrapping. Kidney weights averaged 615 mg. per 100 Gm. of body weight. On histological examination, kidneys showed more severe lesions of nephrosclerosis outside than inside the silk wrapping.

Rats with Unilateral Wrapping Without Contralateral Nephrectomy

Kidneys of eight hypertensive rats were tested between the tenth and fifty-second day after operation. Four kidneys transplanted without ischemia gave variable responses either pressor or depressor (average -1.2 mm. Hg). Four kidneys transplanted with ischemia gave pressor responses lower than normal (average + 7.8 mm. Hg) (fig. 8). In contrast with the previous observation, most of the
renal tissue remained inside the wrapping. Weights of the wrapped and the contralateral kidneys were, respectively, 289 mg. and 515 mg. per 100 Gm. of body weight. Both showed lesions of nephrosclerosis.

Effects of Infarcted and Untouched Contralateral Kidneys

This is an extension of previous studies in which such kidneys were made ischemic during the transplantation procedure. In the present experiments, grafting was performed without interruption of the renal circulation. Unilateral partial renal infarction was done in 13 rats. Kidneys were tested between the eighteenth and forty-seventh day after operation. Grafting of seven infarcted kidneys caused small depressor or pressor responses which averaged +3.5 mm. Hg (range −5 to +9 mm. Hg). Similarly, grafting of six kidneys contralateral to infarcted kidneys had only small effects on the blood pressure of the recipient; changes ranged from −2 to +7 mm. Hg. In two rats, in which responses were +4 and −3 mm. Hg, subsequent grafting of a normal kidney caused blood pressure elevations of +15 and +18 mm. Hg, respectively.

Discussion

The present method of renal transplantation, adapted from those devised by Brull in dogs and Prado and Valle in rats, has the advantage over our previous procedure of avoiding interruption of the renal circulation and of reducing kidney manipulations, factors known to provoke or enhance renin release. Therefore, the demonstration of pressor activity in the renal vein effluent of normal kidneys following grafting onto 24-hour nephrectomized rats indicates the existence of a normal, possibly physiological, release. The gradual rise in pressure further suggests an active secretion. Renin, which is normally present in large amounts in the kidney, may

Figure 5
Pressor response elicited by a clipped kidney grafted with ischemia of 10 minutes' duration. Responses to 0.01, 0.02, and 0.04 units of angiotensin (1); grafting (2).

Figure 6
Response caused by an untouched kidney contralateral to a clipped kidney (procedure without ischemia). Responses to 0.01, 0.02, and 0.04 units of angiotensin (1); grafting (2).
Physiologically be supposed to have a greater role in the regulation of blood pressure and/or water and electrolytes than that of possibly participating in the pathogenesis of hypertension; but this has not been convincingly demonstrated.

In previous transplantation experiments, many of the results may be disregarded because of faulty operative procedure or lack of sensitivity of the recipient animal. Thus, the report by Govaerts and Muller, that normal kidneys secrete large amounts of pressor substances when transplanted onto 48-hour nephrectomized dogs, was attributed by Brull and Dumont to a reduction in renal blood flow caused by the use of Payr cannulae. However, Dumont reported later that normal kidneys with a normal blood flow may elicit a rise in pressure. A rise is regularly obtained when hypotension is artificially induced in the recipient animal.

Other evidence in support of a physiological secretion is provided by results from determinations of renin in blood. Vasopressor material, present in normal rats and dogs, disappears following bilateral nephrectomy; it is increased in hemorrhagic shock. It should be recognized, however, that the methods used involving chemical extraction and biological assay lack specificity, sensitivity, or both, so that the results are only suggestive.

Against the notion of physiological secretion of renin is the observation that anti-renin in amounts sufficient to neutralize all circulating renin does not lower blood pressure in normal dogs; but neither does adrenal medullectomy, although there is agreement that adrenal medulla secretes epinephrine under normal conditions and intervenes at least partly in the regulation of blood pressure.

The present experiments on hypertensive rats confirm and extend our previous observations on infarcted kidneys. In short, kidneys from rats with renal hypertension appear to release variable amounts of renin depending on the method used to produce hypertension. The release is normal, or slightly above normal, in clipped kidneys; it is low or absent in wrapped or infarcted kidneys, as well as in untouched kidneys contralateral to the operated side. These results are not signifi-
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significantly influenced by the operative grafting procedure, although the release has a tendency to be slightly higher when transplantation is associated with a period of ischemia. However, the fact that renin was absent from wrapped and infarcted kidneys as well as from untouched contralateral kidneys, even when transplantation was performed with ischemia, becomes more significant since ischemia is known to promote or increase renin release.

Three explanations may explain the differences in amounts of renin released by the various manipulated kidneys. The first is that the mechanism responsible for hypertension following clipping of the renal artery is different from that obtained in wrapped and infarcted kidneys. There would be two types of renal hypertension: one caused by mechanical reduction of renal blood flow and the other due to wrapping, infarction, or application of a thread around the kidney in the form of a figure-of-8.

The second explanation is that renin overproduction during the acute stage of hypertension leads to partial or complete exhaustion. From this, we would expect that decrease in renin secretions would be more marked whenever severe hypertension develops rapidly. This is the opposite of what we observed; kidneys from rats with early and severe hypertension produced by partial clamping of the renal artery released normal amounts of renin.

The third explanation is based on the assumption that hypertension reduces the activity of the cells responsible for the secretion of the pressor agent, renin, as suggested by experiments in which pressure was raised artificially in the renal arterial tree. Disappearance of renin secretions in wrapped and infarcted kidneys, as well as in contralateral untouched kidneys, would result from high intrarenal arterial pressure, against which the clipped kidney would be protected.

Variations in renin secretions parallel renal lesions which seem also to be dependent on hypertension. Nephrosclerosis is present in the wrapped and infarcted kidneys and in the untouched kidneys, but not in a clipped or "endocrine" kidney. If the third explanation is accepted, it then appears that renal vascular lesions may not perpetuate hypertension through an increased release of renin. Some reports are compatible with this observation. Thus, either no increase, or absence of pressor substances has been reported in the blood of rats with perinephritis, infarction, or application of a figure-of-8, or of dogs and rabbits with partial clamping of the renal artery. The fact that renin is released during acute renal ischemia does not constitute sufficient evidence that it is the primary factor initiating mechanisms responsible for maintenance of hypertension.

Continuous infusion of renin to rabbits for 15 days did not elicit subsequently self-sustained hypertension.

Peart has recently published a "biased" review on renal hypertension. If "bias" is allowable in this subject, it is perhaps appropriate to draw analogies between kidneys and pancreas. If glucagon, a hyperglycemic hormone, had been discovered before insulin, glucagon hypersecretion might have been strongly favored as a cause of diabetes. It is possible that most investigations on the pathogenic role of renin in hypertension have been equally misleading and did not take account of some entirely different mechanism.

Summary

A method is described for grafting kidneys on bilaterally nephrectomized rats without interrupting renal blood flow. Under these conditions, normal kidneys caused a pressor response characterized by a gradual rise in pressure suggesting a continuous endocrine secretion. Kidneys from rats made hypertensive by unilateral clipping of the renal artery, renal encapsulation, or partial infarction were tested during the acute and chronic stages of the experimental disease. Wrapped and infarcted kidneys, as well as the untouched contralateral organs, released little or no pressor substances. On the other hand, clipped kidneys released normal or slightly greater amounts of pressor sub-

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stances; the contralateral untouched kidney did not elicit any pressor effect. Morphological examination showed a relationship between presence of nephrosclerotic lesions and absence of, or decrease of, reninlike pressor substance released. Both changes are believed to be due to high renal vascular pressure against which the partly clipped kidney is protected.

These observations suggest that, if renin is a primary factor in the pathogenesis of renal hypertension, hypertension due to renal artery constriction has a mechanism different from that caused by renal manipulation or infarction.

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