Vasopressor Material in Experimental Renal Hypertension

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Hypertension can be induced readily in rats by a variety of techniques which produce renal injury. However, the pathogenesis of the high blood pressure has not yet been clearly established. One possibility is that a renal humoral pressor mechanism is responsible for the hypertension and the present study was undertaken to test the validity of this thesis.

Methods

PRODUCTION OF HYPERTENSION

White male rats weighing about 150 to 200 g were used. These were rendered hypertensive through bilateral renal injury consisting of clips or wire ties placed on the main renal arteries, ligation of a branch of each main renal artery, or external compression by the figure-of-eight thread. Sixty-five animals were subjected to the clip technique, fifty-eight to ligation technique, and thirty-four to the figure-of-eight. The last method did not yield hypertension with the same consistency as the other two techniques hence was not employed as extensively.

METHOD OF ASSAY

Hypertensive rats were tested for the presence of vasopressor material by injecting 1 cc of their blood into the circulation of normal rats. The latter weighed about 180 g and were anesthetized with 2% pentobarbital sodium given intraperitoneally in dosage of 0.2 cc/100 g body weight. Number-50 polyethylene catheters were inserted into the femoral artery and vein and the arterial catheter was connected to a mercury manometer. Each recipient was given an additional small amount of pentobarbital sodium prior to receiving hypertensive blood in order to provide a fairly uniform, moderately deep grade of anesthesia. Donor blood was injected only when the blood pressure of the recipient was stable over a period of several minutes. Totally nephrectomized rats were employed as recipients in trial runs and seemed more reactive to hypertensive blood than intact rats but the response to normal blood was also correspondingly greater. For this reason, the nephrectomized rat as a test recipient was abandoned in favor of the normal rat.

Recipient rats prepared in the above manner gave a 10 to 15 mm Hg rise in blood pressure on injection of 0.005 µg angiotensin and a rise of 8 to 10 mm Hg on injection of 0.01 µg norepinephrine.

Prior to testing, the mean blood pressure of all donor animals was recorded with a Hg manometer from a catheter in the femoral artery. One cc of blood was then withdrawn from this artery and injected promptly into the femoral vein of the recipient. Injection was completed in 45 seconds. Response of the recipient was measured in mm Hg rise in blood pressure. The result was recorded either with a Statham gauge manometer on a Grass polygraph or was read directly from a Hg manometer.

As a control for each sample of hypertensive blood 1 cc of blood from a normotensive rat was injected into the same recipient. The latter was either a normal animal or one subjected to sham renal operation. There were 55 sham operated rats. Usually the hypertensive blood was given first, followed by the control. The order was reversed in many instances and seemed to make no difference in the results. Each recipient rat was limited to two injections, about 5 to 10 minutes apart, i.e., one from the hypertensive animal and one from the normotensive control. The blood pressures of the control rats and also of the recipient rats ranged from 94 to 126 mm Hg at the time of the test.

Hypertensive rats were assayed for vasopressor material at periods from a few hours to five months after renal injury. Some animals were tested only once, while others were tested two or three times at successive intervals in both the acute and chronic stages of hypertension. A total of 179 tests was performed on 157 rats.

The blood pressures of rats tested during the first two weeks after renal injury ranged from 150 to 210 mm Hg. Although the pressures were labile during this period, they were generally increasing progressively. In the chronic phase of...
hypertension (one to five months), the blood pressure of most animals tended to be more stable at a level of about 200 mm Hg.

Results

Recipients reacted fairly uniformly and in a virtually identical manner to injection of 1 cc of normotensive blood from either a normal or sham operated rat. Of the total of 157 injections, 90% yielded a rise in blood pressure of 0 to 6 mm Hg. Forty per cent of these bloods gave no rise in pressure at all. On the other hand, if the whole hypertensive population is considered, the injection of blood from hypertensive rats frequently produced a distinct pressor response in the recipient. However, positive responses were obtained only during the first two weeks following renal injury. The results of assays for vasopressor material suggested a decline in activity toward the end of the second week of the hypertensive state and after the second week the assays were almost uniformly negative. This difference in vasopressor response permitted a division of renal hypertension into a positive "acute" stage, i.e., up to two weeks following renal injury, and a negative "chronic" stage, i.e., beyond two weeks.

ACUTE HYPERTENSION

Figure 1 represents the response of 119 rats made hypertensive by all three techniques and examined for vasopressor material during the first two weeks after renal injury. The increment in pressor response in mm Hg of hypertensive over normotensive control blood was obtained for each recipient by subtraction and the values were then plotted against the corresponding number of tests. Thus 33 acute hypertensive bloods elicited a response 5 to 9 mm Hg greater than the corresponding controls, 31 gave a response 10 to 14 mm greater, 13 a response 15 to 19 mm greater, etc. In a typical response, elevation of pressure began promptly, i.e., within several seconds after injection of hypertensive blood was started, reached a peak within one minute and then returned gradually to the initial baseline in 5 to 8 minutes. Although the rise in blood pressure varied in magnitude (10 to 44 mm Hg), in most experiments it clearly exceeded the rise (or lack of rise) produced by control blood (fig. 2). In 31 of the 119 assays the rise in pressure yielded by hypertensive blood was only 0 to 4 mm Hg greater than the corresponding control. Only one hypertensive rat yielded a smaller response than the control and this is indicated in figure 1 by a negative increment.

CHRONIC HYPERTENSION

Figure 3 shows the increments in pressor response of hypertensive over normotensive control bloods when hypertension was present for two weeks to 5 months after renal injury. Of a total of 53 "chronic" hyperten-
FIGURE 3
Increments in pressor response of hypertensive versus normotensive blood during chronic renal hypertension (53 tests). Note difference in abscissa values of pressure in contrast to figure 1.

Discussion

Blaquier, Bohr, and Hoobler used the technique of cross transfusion to detect vasopressor material in the blood of rats with renal hypertension. Circulating humoral material was demonstrated in animals which developed hypertension within minutes after release of clamps occluding both renal pedicles. However, rats examined 7 to 38 days after production of renal ischemia showed no such humoral activity, and rats tested 12 to 18 weeks after figure-of-eight ligation were also negative. Apparently no tests were done on hypertensive rats during the first week after renal artery ligation or the figure-of-eight procedure.

Omae, Masson, and Page studied the release of renin in hypertensive rats by placing the injured kidneys into the circulation of nephrectomized recipient animals. Renin released from kidneys with clip on the main renal artery was normal in amount or slightly elevated, whereas it was low or absent from infarcted or externally wrapped kidneys. From these results the authors concluded that renal hypertension might have different origins depending upon the type of kidney injury.

In this study, the results indicated that during the first two weeks following renal manipulation there appears to be a substance in the arterial blood of hypertensive rats which causes a rise in blood pressure when injected into normal rats. After approximately two weeks, however, this technique failed to demonstrate any vasopressor effect, even though the donors in this "chronic" group had much higher blood pressures than those tested in the first two weeks. Also animals with positive assays in the early phase of hypertension were negative on repeat test in the chronic phase.

Positive tests were obtained with acute donor animals which had been subjected to renal artery clips, ligation of renal arteries.
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or external renal compression. Thus three different forms of renal injury yielded similar results. The pressor substance in the blood presumably was the result of the kidney manipulation which produced renal ischemia and/or parenchymal damage. Our study throws no light on its identity or precise mode of production.

Although our method of assay detected vasopressor substance in the blood of most rats with acute renal hypertension, there were some animals with high blood pressure that gave either negative or equivocal results and the reason for this is not clear. It may relate to a lack of sensitivity on the part of some recipient animals or to a low level of vasopressor material in the circulation of the hypertensive animal at the time blood was withdrawn for testing.

In positive assays the magnitude of the vasopressor response induced in recipient rats could not be correlated in any precise manner with the height of the blood pressure of the donor animal. For example, blood from rats whose pressure was 150 mm Hg sometimes yielded a greater response than was obtained from donor animals with a pressure of 180 mm Hg.

The rats in our study usually developed hypertension promptly, i.e., within several hours after renal injury, and the development of high blood pressure coincided with the appearance of vasoconstrictor substance in the blood. Thus a causal relationship is likely, and our results are consistent with the view that renal hypertension arises on a functional basis from a humoral vasoconstrictor mechanism. This would not necessarily exclude other factors from participating in pathogenesis, either in conjunction with a humoral substance or independent of it.

It is of interest that vasoconstrictor activity was demonstrated only in early or acute renal hypertension. Two weeks after renal injury, the blood no longer contained such vasopressor activity. Perhaps the amount of vasopressor substance being elaborated at this time was so reduced that our method of assay failed to detect it. If the peripheral vessels were in some way rendered more sensitive to a vasoconstrictive agent during acute hypertension, they might continue to respond to smaller amounts of such an agent but this amount could be too small for detection by our assay method. Another possibility is the development by the animals of a mechanism which modified or largely destroyed the vasoactive material.

However, the failure to detect such material beyond two weeks suggests it is no longer being produced. One would then conclude that chronic renal hypertension in the rat is not due to a humoral mechanism, but is maintained by other means. Ogden, Collings and Sapirstein suggested that the vasopressor substance present in acute renal hypertension was supplanted subsequently by a neurogenic mechanism. Other factors which might sustain hypertension in the absence of specific humoral activity are an altered electrolyte content of the peripheral vessels, and/or the development of organic peripheral vascular disease.

Summary

Transfer of blood from rats with acute renal hypertension to normal recipient rats demonstrated that a vasopressor substance was present in the circulation of the hypertensive animals. This is consistent with the view that a humoral mechanism plays a role in the origin of the hypertension. In contrast to the acute stage, no vasoactive material could be demonstrated in the blood after the second week of hypertension. Either the amount of pressor substance was too small to detect or none was being produced. In the latter case, chronic renal hypertension cannot be attributed to a humoral mechanism.

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