Renal Medulla and Renoprival Hypertension

RELATIONSHIP BETWEEN CORTICORENAL (RENIN) AND MEDULLORENAL EXTRACTS

By E. E. Muirhead, M.D., and Mary Kosinski, M.S.

With the assistance of B. Brooks

Nephrectomized dogs given sodium parenterally and protein by diet develop acute hypertensive cardiovascular disease.1,2 Protection against acute renoprival hypertension of this type has been demonstrated for (a) intact renal tissue as exemplified by ureterocaval anastomosis,3 (b) autoexplanation of whole kidney or renal medulla,4 and (c) a crude extract of renal medulla.5 Both medullorenal extract and renal tissue without an excretory outlet protect also against the acceleration of renoprival hypertension due to large sodium loads in the absence of food intake.6,7

These observations have been the basis for the proposal that the protective action of the kidney against hypertension appears to be due mainly to a medullary function and that this function may be mediated by an extractable humoral factor.4,5

The present study extends observations on the medullorenal extract in renoprival hypertension of the dog by evaluating its immediate effect on the arterial pressure, by determining whether it possesses an antirenin effect, and by evaluating the role of its protective action against renoprival hypertension when renin is superimposed. The material has appeared in part as an abstract.8

Methods

Adult mongrel dogs were used. The mean arterial pressure was determined without anesthesia by means of direct femoral artery puncture (20-gauge needle) and a mercury manometer. The control mean arterial pressure was determined over four to seven days prior to the nephrectomies after the animal had become accustomed to the procedure. The average of these control values, which usually varied between 110 and 120 mm. Hg, was considered the zero value. The nephrectomies were performed under ether anesthesia at one sitting and required about 20 minutes. Each experiment lasted four days after the renal ablation; no dialysis was interposed. Each day each animal received a diet containing 3 Gm. of protein (casein) per Kg. and about 25 calories per Kg., derived from the protein and additional glucose and peanut oil. Water-soluble vitamins and distilled water (5 ml./Kg.) made up the remainder of the dietary formula. In addition, each day 2.5 mEq. of sodium per Kg. were infused intravenously as physiological saline. Hereafter the use of the term "renoprival hypertension" in this presentation will refer to the acute hypertension of the dog which occurs after renal ablation when parenteral sodium and/or dietary protein are included in the management.

The medullorenal extract was prepared as previously described.5 The corticorenal extract having renin activity was prepared in a similar manner. The outer half of the cortex of fresh canine kidneys was dissected away and placed in cold Geys solution containing 1 per cent urea. The cortex was homogenized in the cold in a Waring blender (10 Gm. per 100 ml. of solution), and the debris was centrifuged out in a refrigerated centrifuge. The final extract was stored at —20 C.

Extracts of fresh lung, skeletal muscle, and erythrocytes, used as controls, were prepared precisely as were the medullorenal and cortical extracts.

The extracts were infused rapidly (three to five minutes) intravenously once daily. The amount of sodium in a given extract was taken into consideration, and the volume of saline infused was lowered and replaced by dextrose in water so that the total daily sodium and volume intake remained constant for all groups.

There were five groups of dogs. Group I (seven dogs) had renal ablation, protein diet, and saline and, in addition, received the corticorenal extract (crude renin). The dose of
cortical extract was 5 ml./Kg./day for two dogs. These animals succumbed in three days. For the remaining dogs of this group, it was found that a dose of 1.5 ml./Kg./day gave a prominent pressor effect, and the animals lived reasonably preserved for the four days of the experiment.

Group II (eight dogs) was treated in the same manner as group I except that the corticorenal extract (renin) was mixed with an extract of nonrenal tissue (lung, skeletal muscle, or erythrocytes), and the mixture was infused. The amounts of cortical and nonrenal tissue extract infused were 1.4 and 5.5 ml./Kg./day, respectively.

Group III (seven dogs) was a normal group (intact kidneys) receiving the same diet, saline, and schedule of corticorenal extract as group I. Three animals of this group had a unilateral nephrectomy prior to the experiment.

Group IV (10 dogs) consisted of nephrectomized dogs which were treated in the same manner as group I except that the cortical extract (renin) was mixed with the medullorenal extract (about 5 ml./Kg./day), and the mixture was infused. For one dog 5 ml./Kg. of the cortical extract were used; for the remainder 1.5 ml./Kg. were used. On three occasions the mixture of extracts was allowed to stand at room temperature for 30 to 60 minutes before infusion. In the remainder of the experiments, the mixture was prepared and immediately infused.

Group V (42 dogs) consisted of an earlier group plus recent additions which were subjected to nephrectomy, diet, and saline and received no extract.

The mean arterial pressure was determined before and five to 15 minutes after the infusion of extract or extracts and about 40 minutes and 15 and 24 hours later.

The regression line was determined for the change in arterial pressure from the control after the renin effect had passed. It was computed in order to determine whether this slope differed from zero. The elevation of the arterial pressure on the fourth day was determined from the estimating equation and was found to be similar to the average change in pressure on the same day. The change from the control of the arterial pressure for each day within the various groups was compared by means of the t-test.

Results

IMMEDIATE EFFECT OF MEDULLORENAL EXTRACT

The mean arterial pressure was determined before and five to 15 minutes after the intravenous infusion of the crude medullary extract on 29 occasions involving 15 dogs. The results are summarized in figure 1.

RENIN PRESSOR EFFECT

The renin pressor response for three groups is summarized in table 1. The elevation of arterial pressure 30 to 45 minutes after infu-
TABLE 1

Average Elevation of Mean Arterial Pressure Five to Fifteen Minutes After Infusion of Corticoenal Extract (Renin) for Three Groups

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>Group I</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>66</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>19</td>
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</tr>
<tr>
<td>60</td>
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<td>53</td>
<td>82</td>
</tr>
</tbody>
</table>

*The "time" represents the time after initiation of the experiment, which for groups I and IV is the same as the time after bilateral nephrectomy. Group I was nephrectomized and received renin, group III was the normal group which received renin, and group IV was nephrectomized and received a mixture of renin and the medullorenal extract.*

...baseline value. Thus, renoprival hypertension evolved in the face of daily renin pressor effect. The average elevation of the mean arterial pressure by the fourth day for this group was +41 mm Hg. By means of the estimating equation the fourth day pressure change was found to be +42 mm Hg. The slope of the change differed from zero (p < 0.001). The severity of the hypertension was not different from that of group V which had nephrectomy, the same diet and sodium intake, but no superimposed renin (p = 0.55).

The results for the various groups are summarized in figures 2, 3, and 4 and table 2.

GROUP II (NEPHRECTOMY PLUS RENIN AND A NONRENAL EXTRACT)

The sequence of arterial pressure change was the same for this group as for group I, namely, the postrenin pressure was elevated each day and renoprival hypertension evolved along with daily renin pressor responses. The average elevation of the mean arterial pressure on the fourth day was +33 mm Hg. According to the estimating equation the pressure change for the fourth day was +32 mm Hg. The slope of these data differed from zero (p < 0.001). This group did not differ...
RENOPRIVAL HYPERTENSION

Infusion cortical extract (renin) — No. 1139 Bil. nephrectomy + extract skeletal muscle — No. 1151 Bil. nephrectomy + extract renol medulla

Bil nephrectomy + Mo (2.5 meq./kg./day) + dtelory protein (30 gm/Kg./day)

20 30 00 50 60

Hours

FIGURE 3

The same type of experiment as shown in figure 2. The solid line depicts the pressure changes in a nephrectomized dog receiving daily a mixture of corticorenal extract and an extract of skeletal muscle. The broken line depicts changes in a nephrectomized dog receiving a mixture of corticorenal and medullorenal extracts. The first post-infusion pressure was taken about 25 minutes after the infusion. After 90 hours the difference between the two examples was 40 mm. Hg.

either from group V which had nephrectomy without extract (p.6) or from group I which had nephrectomy and renin (p ~ 0.45).

GROUP III (NORMAL PLUS RENIN)

Each day after the renin effect the arterial pressure returned either to baseline or near this value. The average change of the mean arterial pressure at four days was +2 mm. Hg, while the estimating equation gave a value of +1 mm. Hg for the fourth day. The slope of the data on pressure change did not differ from zero (p.9). Thus, this group did not develop sustained hypertension. The latter was expected from results in the literature. The same results were observed whether both or only one normal kidney was present. The results differed from those of groups I, II, and V.

GROUP IV (NEPHRECTOMY PLUS RENIN AND MEDULLORENAL EXTRACT)

The sequence of arterial pressure change for this group was similar to that of group III. The change of the mean arterial pressure at four days was +4 mm. Hg by averaging the values and +6 mm. Hg from the estimating equation. The slope fitted to the data did not differ from zero (p.65). The same results were observed when the mixture of extracts was infused immediately after mixing as when the mixture was allowed to stand for 30 to 60 minutes before the infusion. This group was different from group I (p < 0.001) but did not differ from group III (p.5).

GROUP V (NEPHRECTOMY WITHOUT EXTRACT)

The average elevation of the mean arterial pressure at four days was +27 mm. Hg. From the estimating equation the change in pressure at four days was +29 mm. Hg. The slope was different from zero (p < 0.001). This figure represents the standard result in this laboratory for nephrectomized dogs receiving dietary protein and parenteral sodium.

Discussion

The medullorenal extract in the doses used did not prevent the exaggerated renin pressor response of nephrectomized dogs, as shown by the significantly lower pressor response in normal dogs receiving renin alone when compared to nephrectomized dogs receiving renin plus medullorenal extract. Moreover, there

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Summary of the Data from the First Four Groups

| Dog no. | Wt. (Kg.) | Control BP (mm. Hg) | Δ BP (mm. Hg) | Day | Group I | Dog no. | Wt. (Kg.) | Control BP (mm. Hg) | Δ BP (mm. Hg) | Day | Group II | Dog no. | Wt. (Kg.) | Control BP (mm. Hg) | Δ BP (mm. Hg) | Day | Group III | Dog no. | Wt. (Kg.) | Control BP (mm. Hg) | Δ BP (mm. Hg) | Day | Group IV | Dog no. | Wt. (Kg.) | Control BP (mm. Hg) | Δ BP (mm. Hg) | Day |
|---------|-----------|---------------------|---------------|-----|---------|---------|-----------|---------------------|---------------|-----|---------|---------|-----------|---------------------|---------------|-----|---------|---------|-----------|---------------------|---------------|-----|---------|---------|-----------|---------------------|---------------|-----|---------|---------|-----------|---------------------|---------------|-----|
| 1111    | (12.0)    | 117                 | +33           |     |         | 1127    | (11.9)    | 104                 | +26           | +39 |         | 1115    | (10.6)    | 123                 | -3            | +4  |         | 1114    | (9.1)     | 122                 | -2            | -7  |
| 1113    | (13.6)    | 120                 | +40           |     |         | 1139    | (14.2)    | 114                 | +34           | +31 |         | 1112a   | (10.4)    | 120                 | -10           | 0   |         | 1116    | (12.3)    | 117                 | +3            | +6  |
| 1120    | (11.4)    | 122                 | +28           | +13 |         | 1145    | (15.6)    | 110                 | +17           | +40 |         | 1112b   | (10.4)    | 129                 | -10           | -10 |         | 1118    | (11.7)    | 120                 | -5            | -5  |
| 1124    | (12.0)    | 100                 | +60           | +60 |         | 1181    | (12.0)    | 117                 | +13           | +13 |         | 1125    | (12.0)    | 122                 | +1            | +18 |         | 1119    | (12.5)    | 120                 | -9            | -5  |
| 1126    | (9.8)     | 109                 | +51           | +71 |         | 1182    | (12.0)    | 112                 | +18           | +48 |         | 1129    | (12.0)    | 129                 | +14           | +1  |         | 1123    | (11.0)    | 120                 | +5            | +15 |
| 1130    | (12.1)    | 104                 | +31           | +31 |         | 1183    | (13.6)    | 117                 | +23           | +28 |         | 1140    | (12.9)    | 150                 | 0             | -5  |         | 1131    | (10.7)    | 117                 | +3            | +11 |
| 1156    | (10.7)    | 95                  | +28           | +30 |         | 1184    | (15.0)    | 112                 | +33           |         |         | 1143    | (12.6)    | 104                 | +16           | +6  |         | 1138    | (15.2)    | 118                 | +17           | +12 |
|         |           |                     |               |     |         | 1186    | (10.3)    | 73                  | +32           | +37 |         |         |           |                     |               |     |         | 1151    | (7.4)     | 109                 | -5            | +4  |
|         |           |                     |               |     |         |         |           |                     |               |     |         | 1152    | (12.4)    | 108                 | +32           | -3  |         | 1155    | (11.3)    | 129                 | 0             | +12 |
| Average |           |                     |               |     |         | 109                 | +39           | +41 |         |         |           |                     |               |     |         | 117     | +3        | +4  |

From estimating equation

<table>
<thead>
<tr>
<th>Slope versus zero--</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
</tr>
</tbody>
</table>

* Wt. Kg. = body weight in Kg.; control BP = control mean arterial pressure; Δ BP = change from control in mean arterial pressure on the third and fourth days. Group I had bilateral nephrectomy plus renin daily; group II had bilateral nephrectomy plus renin and an extract of nonrenal tissue daily; group III was a normal group which received renin daily; and group IV had bilateral nephrectomy plus renin and the medullorenal extract daily.
was no difference in pressor response of nephrectomized dogs receiving renin alone and nephrectomized dogs receiving renin plus medullorenal extract. Thus, the medullorenal extract did not appear to possess an antirenin effect and probably did not have a major antiangiotensin influence in vivo. Tachyphylaxis was not a feature under the circumstances of the experiment.

The change of the mean arterial pressure for groups I, II, and V, the groups developing sustained hypertension, were not significantly different from each other. The superimposition on the renoprival state of prominent daily renin pressor peaks associated with a pressor effect of one to several hours did not seem to magnify renoprival hypertension.

Extract derived from nonrenal tissue, such as lung, skeletal muscle, and erythrocytes, failed to modify the evolution of renoprival hypertension under the conditions of the experiment. It has previously been noted that an extract of urinary bladder also did not prevent renoprival hypertension. These negative results lend emphasis to the antihypertensive effect of the medullorenal extract.

The prevention of sustained hypertension in the renoprival state by the medullorenal extract when renin was superimposed was associated with a pattern of arterial pressure which closely resembled that which appeared when renin alone was given to normal dogs having one or two intact kidneys. The main difference between the two patterns of arterial pressure was due to a lower pressor response for the normal group. Whether this difference could have resulted from a difference in the magnitude and duration of the same function could not be ascertained because of the design of the experiment. The similarity of the arterial pressure sequence of these two groups (III and IV) does suggest a similar type of function being exerted by intact renal tissue and the medullorenal extract.

The material in the medullorenal extract which is active in preventing renoprival hypertension of the dog does not seem to have its effect by directly antagonizing the renin-angiotensin system. Moreover, the active material does not have a sustained, acute depressor effect. Spot check of the crude medullorenal extract revealed no pyrogenic effect in rabbits. The results suggest that the active principle of the medullorenal extract which prevents renoprival hypertension of the dog acts directly on the arterial-arteriolar wall. The nature of such postulated action remains obscure.

**Summary**

A crude extract of renal medulla (medullorenal extract) has been studied in relation to a crude extract of renal cortex which possessed potent renin pressor activity. The medullorenal extract either effected no immediate change in the arterial pressure or caused a mild to moderate pressor effect. The latter was thought to be due to contamination with renin, since precise separation of cortex and medulla may not have been consistent. The medullorenal extract did not display a sustained, acute depressor effect. The extract was not pyrogenic in rabbits.

The production of renin pressor effect in nephrectomized dogs did not alter the evolution of renoprival hypertension. The mixture of renin and nonrenal extracts did not alter the evolution of renoprival hypertension in the dog. The medullorenal extract, on the other hand, prevented renoprival hypertension in the presence of daily renin pressor responses. The latter results were similar to those obtained when renin was infused in normal dogs.

The results suggest that the medullorenal extract protected against renoprival hypertension by a route other than one directly related to the renin-angiotensin system. Such a route could be due to an effect on small arteries and arterioles. The results also are consistent with an active principle in the medullorenal extract which reproduces the function of intact renal tissue.

**References**

1. Muirhead, E. E., Jones, F., and Graham, P.: Hypertension following bilateral nephrectomy of the dog: Influence of dietary protein on
its pathogenesis with emphasis on its development in absence of "extracellular fluid" expansion. Circulation Research 1: 439, 1953.


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