Sustained Depressor Effect of Renal Medullary Extract in the Normotensive Rat

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In addition to its well-known pressor activities, the normal kidney has long been suspected of possessing an antihypertensive endocrine function responsible for its "protective" effect in various forms of experimental hypertension. However, the localization and nature of this effect have proven elusive. The demonstration by Braun-Menendez and von Euler, and Grollman and his co-workers that hypertension develops in the bilaterally nephrectomized animal renewed interest in the possibility that certain forms of hypertension may represent a relative deficiency of a physiologically active depressor material. The observation that hypertension does not develop in the severely uremic animal with uretero-caval anastomosis suggested that the normotensive state of these animals was related to a nonexcretory renal metabolic function. Investigations by Muirhead and his colleagues have implicated the renal medulla as the source of antihypertensive activity, since explants and extracts of medulla appear to prevent the development of renoprival hypertension to a degree comparable with whole kidney explants or homogenous renal transplants. On the other hand, Sokabe and Grollman, on the basis of the effects of medullectomy and corticectomy, have concluded that the maintenance of normotension resides in kidney cortex and not medulla.

In an attempt to investigate the properties of the antihypertensive principle, Hamilton and Grollman have prepared a dialyzable, acetone and butanol-soluble extract from whole kidney which is effective in reducing blood pressure in hypertensive but not normotensive animals. These findings utilizing whole kidney extracts have been confirmed by Milliez and his co-workers. The active principle of renal medulla has been shown by Muirhead et al. to be an ethanol soluble, low molecular weight compound which was not retained on Dowex-50 resin. On the basis of experiments with whole kidney extracts, Gordon has concluded that any renal depressor activity may be associated with its content of adenosine nucleotides, substances known to have a potent depressor effect.

The present study was undertaken in an attempt to determine the separate effects of rabbit kidney cortex and medulla on the blood pressure of normotensive rats and to investigate certain properties of any observed depressor activities.

Methods

Male albino rabbits of the New Zealand-Flemish strain weighing between 3 kg and 4 kg were fed ad libitum on Purina rabbit pellets providing a daily sodium and potassium intake of 10 to 20 mEq and 70 to 80 mEq respectively. They were killed by a blow on the head followed by carotid exsanguination. The kidneys were rapidly removed, decapitated and placed on ice-cold petri dishes for dissection. Each kidney was quartered and the cortex separated from medulla by scissor dissection through the cortico-medullary junction. The medulla represented only inner medulla (papilla) and excised cortex and outer medulla. One to 2 g of medulla were homogenized in 5 ml to 10 ml of ice-cold saline, the homogenate centrifuged in the cold, and the supernate assayed for its effect on the blood pressure of the anesthetized rat. All extracts were prepared from rabbit medulla unless otherwise noted. Crude extracts were also prepared in a similar manner from rabbit kidney cortex, spleen, liver, intestine, and lung.
In certain experiments, various purification and characterization procedures were carried out on the crude homogenates which are described under the appropriate heading in the section on Results. In two sets of experiments, column chromatography of the crude extracts was performed. In the first, Sephadex (G-25) was washed with distilled water and packed in columns to provide an external volume of approximately 14 ml and an internal volume of 20 ml. Crude homogenates of cortex and medulla were chromatographed, eluted with distilled water, and 0.1 ml of 2 ml eluates bio-assayed. In additional experiments, the crude homogenate was first subjected to pressure ultrafiltration through Visking dialysis tubing and the ultrafiltrates then chromatographed on Sephadex. In the second set of experiments, crude homogenates of medulla were chromatographed by gradient elution on diethylaminoethyl ether, (DEAE), Sephadex (G-50). The resin was washed with 0.1 \( \times \) NaOH and distilled water and, after acidification to pH 4.5 with 1 \( \times \) HCl, was rewash with distilled water, 0.1 \( \times \) NaOH and finally with 0.005 \( \times \) Na\(_2\)HPO\(_4\). The resin was pressure packed under 15 pounds nitrogen in a column which was then attached to a reservoir containing 0.005 \( \times \) Na\(_2\)HPO\(_4\). Gradient elution of the crude homogenate (3.8 g medulla/9 ml homogenate) was accomplished with 0.12 \( \times \) Na\(_2\)HPO\(_4\)/0.05 \( \times \) NaH\(_2\)PO\(_4\) and 1.0 \( \times \) NaH\(_2\)PO\(_4\) at 5°C. Eight ml eluate fractions were collected and analyzed for blood pressure effect, eluate pH, and protein by the method of Lowry et al.\(^{13}\) Ultraviolet 260/280 absorption spectra, and pentose sugar by the orcinol reaction\(^{14}\) were analyzed as indication of nucleotide content.

Female Sprague-Dawley rats weighing between 200 g and 250 g were used for bio-assay. They were injected intraperitoneally with pentobarbital (10 mg) and pentolinium (4 mg), tracheotomized and vagotomized. Assay substances were injected into the jugular vein and the mean carotid blood pressure recorded kymographically. A small amount of heparin was injected into the arterial cannula to prevent clotting.

**Results**

**EFFECT OF CRUDE MEDULLARY EXTRACT**

The effect of intravenous injection of crude rabbit medullary homogenate on the blood pressure of the anesthetized rat is illustrated in figure 1. There was a prompt fall in blood pressure which gradually returned to control levels after an average interval of ten minutes. This depressor effect was invariably observed with crude extracts of rabbit medulla while a similarly prepared homogenate of rabbit cortex produced a sustained elevation of blood pressure associated with its renin content. Extracts of spleen, liver, lung or intestine produced either no effect, or a transient rise in rat blood pressure. The prolonged hypotensive effect was also consistently observed in extracts from rat medulla and occasionally in pig and human medullary extracts.

Hypotensive activity of medullary extracts was also observed in the noupentolinium-treated animal although the depression in blood pressure was less sustained. The degree of blood pressure fall was proportional to the amount of substance injected until a maximum depression occurred. This relationship is evident in figure 2. A maximum hypotensive effect occurred in this case at about 45 mg wet weight medulla; further dose increases produced no additional decrement in blood pressure. During repeated intravenous administration of medullary homogenate into the same rat there was no evidence of anaphylaxis or tachyphylaxis.

**CHARACTERIZATION OF CRUDE MEDULLARY EXTRACT**

Studies were undertaken to characterize the substance responsible for the prolonged depressor activity.

**Dialysis Experiments**

A saline homogenate of rabbit medulla was dialyzed for 18 hours against distilled water. The dialysate was evaporated to dryness and restored to the original homogenate volume with saline, and both dialysate and nondialyz-
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FIGURE 2
Dose response of rat blood pressure to a saline homogenate of rabbit kidney medulla. Decrement in blood pressure plotted upward on ordinate. Approximately 1 g medulla/1 ml homogenate. Maximal blood pressure fall occurred at about 0.045 ml. (45 mg wet weight medulla.)

Approximately 1 g medulla/cc saline homogenate

FIGURE 3
Depression of rat blood pressure by a dialysate of rabbit medulla. 2 g medulla/5 ml saline homogenate. 0.1 ml of nondialyzable and dialyzable material injected intravenously. See text for details of preparation. n = 7.

Ethanol Solubility
A saline homogenate of medulla was treated with absolute ethanol to final concentrations between 33% and 90%. After centrifugation, the ethanol supernate was evaporated to dryness and the residue restored to the original homogenate volume with normal saline. The reconstituted saline solution was utilized

*Pan-protease, Worthington.
Depression of rat blood pressure by ethanol extracts of rabbit medulla. See text for details of preparation. 2 g medulla/6 ml saline homogenate. Each dose is equivalent to 0.1 ml crude extract, \( n = 11 \).

Comparison of deproteinized rabbit cortex and medulla with bradykinin and ATP. 25% ethanol extract of 2 g cortex and medulla/6 ml saline homogenate prepared as in figure 4. \( n = 6 \).

Figure 4 illustrates that the depressor activity was ethanol soluble. It is evident that full depressor activity was retained in the ethanol supernate, an activity comparable to the crude homogenate.

Comparison with Bradykinin and ATP

A 25% ethanol extract of cortex and medulla was prepared as described above. Cortical renin pressor activity normally present in the crude homogenate was removed from the supernate by this treatment. It can be seen from figure 5 that quick-acting cortical depressor activity was then found to be present. It is evident that the protracted hypotensive effect of medulla differs from the quicker acting depressions of pressure produced by cortical extract and nucleotides (ATP). In addition, the prolonged pattern of medullary depression did not resemble the quick-acting response of bradykinin. The latter substance was found to have inconsistent effects in the pentolinium-treated rat but when hypotensive activity was present, it was typically short-acting.

COLUMN CHROMATOGRAPHY OF CRUDE MEDULLARY EXTRACT

Figure 6A illustrates the results of Sephadex chromatography of saline homogenates of rabbit cortex. In the external volume of the column which represents the passage of relatively large molecular weight substances, typical renin pressor activity was present (fractions 4 to 6), while in the internal volume, representing relatively small molecular weight substances, short-acting activity resembling pure nucleotides was evident, (fractions 16 to 20). When a similar experiment was carried out with rabbit medulla (fig. 6B), no activity was seen in the external volume. At the beginning of the internal volume (fraction 9), typical protracted depressor activity was present initially which gradually gave way to short-acting responses (fractions 11 to 13). In fraction 9, where only sustained depressor activity was evident, there was no rise in the uv 260/280 absorption ratio above that present in the external volume. However, in fractions 10 to 13, there was a rise in the uv 260/280 absorption ratio in association with
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quick-acting depressor responses typical of nucleotides; it is evident that in fraction 11, peak elevation in uv 260/280 ratio coincided with maximum quick-acting depressor activity. When the crude homogenate was first subjected to pressure ultrafiltration and the filtrate chromatographed on Sephadex, identical patterns of prolonged and quick-acting responses were seen. It seemed likely, therefore, that the kidney medulla might contain at least two relatively small molecular weight substances (mol wt < 4500) with depressor activities of different characteristics.

In an effort to separate such activities more distinctly, a saline homogenate of medulla was column-chromatographed by gradient elution on DEAE-Sephadex, which serves both as an anion exchange resin and as a molecular sieve (fig. 7). In this experiment, nonspecific depressor activity was present in the first 23 fractions associated with the passage of initial buffer through the resin. Prolonged depressor activity, characteristic of that in crude homogenates, was present in fractions 24 to 29; this was associated with a slight rise in uv 260/280 ratio, orcinol reactive material and protein concentration. Fractions 30 to 39 were devoid of activity on blood pressure, but displayed considerable amounts of protein. Short-acting depressor activity typical of nucleotides was present in fractions 40 to 55 associated with a sharp rise in uv 260/280 ratio and pentose sugar in addition to a further elevation in protein. Fractions 55 to 75 represented nonphysiological depressor activity which was found with the injection of acid buffer alone. It seemed clear therefore that kidney medulla contained at least two depressor activities: one, a short-acting activity probably associated with its nucleotide content; the other, a prolonged activity representing an unidentified substance(s) probably nonnucleotide in nature.

**Discussion**

The present study demonstrates that there is a nonnucleotide substance(s) in kidney medulla which possesses sustained depressor activity in the normotensive rat. This activity has not been found in kidney cortex or in certain nonrenal tissues, although both medullary and renin-free cortical homogenates possess short-activity depressor activity associated with their nucleotide content. The presence of nucleotide-type activity in cortex and medulla confirms similar findings for whole kidney homogenates by Gordon,11 who has concluded that the depressor action of rabbit kidney extracts is due to its adenosine nucleotide content. It would appear unlikely, however, that any physiological renal control in lowering peripheral arteriolar resistance would primarily reside in the various nucleotide fractions since their depressor effects are so transient and since they are widely distributed in many tissues other than kidney.

For many years investigations have been made of the effects of whole kidney extracts in hypertensive animals and humans.8,9,15-22 Any depressor effects of parenteral extracts in humans have been interpreted as secondary to the febrile, hypersensitive, and at times shock-like reactions which accompany such administration.19,20 Grollman and his co-workers have shown that oral administration of a dialyzable extract of whole kidney to hypertensive animals and humans will result in sustained blood pressure depression,15 but interpretation as to its specificity has proven difficult. Although whole kidney extracts have
Characteristics of Medullary Depressor Substance

1. Prolonged depressor activity.
2. Dose-dependent blood pressure fall to a limiting maximum.
3. Dialyzable.
4. Ethanol soluble.
5. Nonprotein.
6. Resistant to peptide hydrolases.
7. Destroyed by heating at 100°C for one hour.
8. Molecular weight <4500.
9. Nonnucleotide chemical and physiological characteristics.

Consistently lowered the blood pressure of hypertensive animals; no effect has been noted in normotensive animals. Similarly, the medullo-renal extract of Muirhead et al., active in the prevention of renoprival hypertension, fails to show an immediate acute depressor effect until renoprival hypertension has become well established. The finding in the present study that medullary homogenate produces a sustained depressor effect in normotensive rats may result from the fact that the assay animal was vagotomized and pretreated with pentobarbital and pentolinium, procedures which result in a relatively basal blood pressure state and which tend to minimize adaptive pressor responses to the introduction of a depressor agent. There was no evidence that the medullary depressor effect represented a hypersensitivity reaction since the animals tolerated repeated injections remarkably well over periods of several hours without evidence of tachyphylaxis or anaphylactic reactions. Muirhead and his co-workers have also observed parenteral injection of medulla to be unusually free from hypersensitive or pyrogenic reactions.

Table 1 summarizes our knowledge concerning the properties of sustained medullary depressor activity. These properties bear a close resemblance to the medullo-renal extract of Muirhead et al. which has been shown to be experimentally effective in the prevention of renoprival hypertension. In addition, neither the activity of the present medullary depressor substance(s), nor the principle active against renoprival hypertension have been found in kidney cortex or nonrenal tissues. Although it must be emphasized that little is known regarding any physiological control which sustained medullary depressor activity may exert in normotensive or experimentally hypertensive animals, the similarities of the present medullary depressor substance(s) and the principle active in renoprival hypertension are noteworthy.

Many of the problems in attempting localization of the antihypertensive endocrine function of kidney are a result of the extremely varied and difficult experimental designs which must necessarily be employed with present knowledge and techniques. Therefore, although it is of considerable interest that the kidney medulla produces sustained blood pressure depression in the normotensive rat, the failure to find significant depressor activity in extracts of kidney cortex does not rule out an antihypertensive role of cortex upon induction of experimental hypertension. Furthermore, it must be re-emphasized that the various forms of experimental hypertension do not necessarily reflect a common pathogenesis, and the failure of certain investigators to find cortical depressor activity in renoprival hypertension is not contradictory to the inability of others to observe medullary depressor activity in hypertension of different experimental design.

Little is known of the mechanism by which the fall in blood pressure occurs in either hypertensive or normotensive animals treated with kidney extracts. In the present study, administration of crude medullary extract resulted in blood pressure depression in the presence of excess pentolinium which suggests that the hypotensive action is not mediated through the autonomic nervous system. It will require much additional investigation to determine the mechanism of action of sustained medullary depressor activity and whether this action represents a physiologically significant substance or a nonspecific depressor effect.
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Summary
Crude homogenates of rabbit medulla produced a sustained depression of blood pressure when injected intravenously into the normotensive, anesthetized, pentolinium-treated rat. Similar activity was present in rat medulla, but not in extracts of renin-free cortex or certain nonrenal tissues.

The sustained depressor activity of medullary extract possessed different physiological and chemical characteristics from the short-acting nucleotide depressor activity which is present in both cortical and medullary extracts. In addition, prolonged medullary depressor activity was separable from medullary nucleotide activity by column chromatography.

The activity responsible for sustained depressor activity was a dialyzable, ethanol soluble, low molecular weight (<4500 mol wt) substance(s) which was resistant to a mixture of peptide hydrolases and destroyed by heating for one hour at 100°C.

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