Adrenal Cortex and Renal Pressor Function

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The concept of an endocrine relationship between adrenals and kidneys has recently received considerable support. Adrenalectomy causes an increase in granularity of the juxtaglomerular cells and in pressor substances contained in kidneys; desoxycorticosterone has the opposite effects while cortisone and cortisol are reported, without supporting data, to have no effects.

It is not clear what these results mean in the absence of direct evidence that morphology of the juxtaglomerular cells and hormonal content accurately reflect secretion. The purpose of the present experiments was to determine the pressor activity of the renal vein effluent during adrenal insufficiency and hyperactivity, and to explore by sequential determinations of pressor substances contained and released by the kidney during desoxycorticosterone treatment, the validity of the assumption that renin content is an index of the renal pressor function.

Whenever used, the term renin refers, as originally described by Tigerstedt and Bergman, to the substance or substances which elicit a prolonged and sustained pressor effect when kidney extracts are administered intravenously to bilaterally nephrectomized animals.

Methods

Sprague-Dawley rats weighing 180-225 g were used. In the first series of experiments, males were bilaterally adrenalectomized and divided into three groups. In group I, 20 rats were kept without treatment; in group II, 12 rats received 1% NaCl as drinking fluid, and in group III, 14 rats were maintained on a daily dose of 0.2 mg of cortisol acetate. Renal pressor substances were determined between the 2nd and 7th day in group I, between the 10th and 23rd day in group II, and between the 6th and 19th day in group III. In addition, similar determinations were carried out in seven normal rats, which were used as controls.

In a second series, nine rats received 5 mg/day of cortisol acetate and 17 rats, 5 mg of cortisol acetate. Six rats were used as controls. Blood pressure was recorded by tail sphygmography. Kidneys were tested for pressor substances between the 1st and 3rd week.

In a third series, 12 female rats received a gel preparation of ACTH at a daily dose of six units given in two subcutaneous injections while six rats were used as controls. Blood pressure was measured. Animals were tested between the 10th and 22nd day. At autopsy, adrenals were removed and weighed.

In a fourth series, female rats were unilaterally nephrectomized and divided into four groups consisting respectively of 31, 15, 23, and six animals treated as follows: group I, desoxycorticosterone acetate (DCA) 10 mg/day subcutaneously and 1% saline as drinking fluid; group II, 1% saline; group III, DCA 10 mg/day plus tap water and group IV, tap water. Blood pressure was regularly recorded. Kidneys from two to five animals in each group were tested at intervals between the 1st and 25th day. Some of the kidneys removed at the time of nephrectomy were also used for determination of renin content; the data obtained were added to those of the control group.

Determination of pressor substances released into the renal vein was done by grafting kidneys onto 24-hour nephrectomized rats and using the height of the pressor response as index of secretion. The method consisted of the following steps: heparinization of both donor and recipient (2 mg intravenously), cannulation of the renal vein and artery, perfusion with saline to remove blood, and anastomosis with femoral vein and artery of the nephrectomized recipient. Interruption of the renal circulation was kept at about 10 minutes. This method was preferred to another which does not involve interruption of the renal circulation, because of the sharper and more clearly defined end-point of the pressor response. Although the second method appears to be more physiological, we have shown that under the same experimental conditions, both methods gave similar results.

Pressor substances contained in kidneys were determined by a direct and indirect method. The
ADRENAL CORTEX AND RENAL PRESSOR FUNCTION

TABLE 1

Effects of Adrenalectomy on the Index of Renin Content and the Index of Renin Secretion

<table>
<thead>
<tr>
<th></th>
<th>Adrenalectomy</th>
<th>Adrenalectomy + 1% NaCl</th>
<th>Adrenalectomy + Cortisone 0.2 mg/day</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index of renin secretion in mm Hg</td>
<td>22.3 ± 2.2*</td>
<td>28.8 ± 1.6</td>
<td>29.5 ± 1.8</td>
<td>22.4 ± 1.5</td>
</tr>
<tr>
<td>Index of renin content in mm Hg</td>
<td>23.3 ± 1.3</td>
<td>33.0 ± 2.0</td>
<td>..</td>
<td>19.1 ± 0.6</td>
</tr>
</tbody>
</table>

*Mean ± standard error of the mean.

The indirect method specific for renin consisted of incubating kidney extracts with renin-substrate and measuring the amount of angiotensin formed by assay on the guinea pig ileum. The kidney extract prepared above was acidified to pH 3.8, incubated at 37° C during 30 minutes, brought to pH 7.4, and centrifuged. Incubation was carried out at 37° C during one hour in tubes containing 1 ml of the supernatant, 50 mg of dried horse plasma as substrate dissolved in 0.5 ml of phosphate buffer pH 7.4 and merthiolate. Without further treatment, the incubate was tested on the guinea pig ileum by matching each unknown against standard doses of isoleucine-5-angiotensin II. The results are expressed in µg of angiotensin released by the enzyme renin contained in one gram of kidney.

Results

EFFECTS OF ADRENALECTOMY (table 1)

Pressor responses to 10 renal grafts from adrenalectomized rats averaged 22.3 mm Hg, a value which is not significantly different from the control average of 22.4 mm Hg. However, examination of sequential data indicated a slight fall during the first four days post-adrenalectomy (average 17.2 mm Hg) followed by an increase above normal between the 5th and 7th day (average 27.6 mm Hg). Such studies were limited by the low resistance of the animals to the operative procedure associated with renal grafting, especially at a time when changes would presumably be the most pronounced. This latter increase in the index of renin secretion was confirmed and found to be statistically significant in nine rats given 1% saline to drink (P value between 0.01 and 0.02) and in 16 rats which received a maintenance dose of cortisone (P value less than 0.01).

The index of renin content of kidneys from 18 untreated adrenalectomized rats was slightly but not significantly increased. However, when rats were kept alive by addition of salt to their drinking fluid, this increase became highly significant (P value less than 0.01). The increases in both indices (secretion and content) found in adrenalectomized rats given salt or cortisone are probably due to the longer period of survival and not to the treatment per se, since, as shown later, administration of salt or cortisone to normal rats causes no change in renin content and a decrease in secretion.

EFFECTS OF CORTISONE AND CORTISOL

Administration of cortisone to normal rats at the daily dose of 5 mg over periods from one to three weeks caused some weight loss and slight hypertension. Blood pressure increased from an average of 115 mm Hg (range 105-130) to 148 mm Hg (range 120-165). The index of renin secretion from nine kidneys averaged 16.9 ± 1.6 mm Hg as compared with 24.7 ± 1.8 mm Hg in control rats; this difference is highly significant (P less than 0.01). Values for the index of renin content remained within the normal range with an average of 20.0 ± 0.7 mm Hg. Similar results were obtained in 17 rats treated with cortisol which also developed mild hypertension. The index of renin secretion
was significantly decreased with an average value of 15.0 ± 1.2 mm Hg (P less than 0.01), while the index of renin content remained normal, averaging 23.9 ± 1.6 mm Hg. Thus, cortisone and cortisol partially inhibited secretion of renal pressor substances without affecting the content in kidneys. Microscopic examination of heart, kidney, and mesentery did not show vascular lesions. No correlation could be established between blood pressure and renin secretion.

**EFFECTS OF ACTH**

The daily injections of six units inhibited body growth and stimulated adrenal weight. Adrenal weight varied between 64 and 134 mg as compared with a range of 35 to 46 mg in control animals of similar size. There was no change in blood pressure. The index of secretion of renal pressor substances was normal, averaging 20.9 ± 1.6 mm Hg as compared with 21.4 ± 2.1 mm Hg in controls. The same situation obtained for the index of renin content of kidneys which averaged 28.4 ± 1.5 mm Hg in experimental and 25.5 ± 2.0 mm Hg in control animals.

**EFFECTS OF DESOXYCORTICOSTERONE**

In the control rats given tap water (group IV), renin content and secretion remained quite constant during the course of the experiments. Results from each set of determinations were averaged and used for comparison. Pressor responses to 10 renal grafts averaged 22.6 ± 1.8 mm Hg. The pressor activity of 16 saline extracts averaged 23.2 ± 0.9 mm Hg. Twenty-two extracts of normal kidneys were examined for their ability to release angiotensin on incubation with renin substrate. Twenty-one of them released...
0.3 \( \mu g \) and one 0.6 \( \mu g \) of angiotensin per gram of kidney.

Administration of DCA plus salt (group I) caused a significant rise in blood pressure around the 11th day so that between the 13th and 25th day all the rats tested were hypertensive (fig. 1A). Renal secretions were first affected, decreasing within a few days to insignificant levels. Kidneys grafted on the 5th, 11th, and 18th day either did not elicit any pressor response in the recipient rat or caused a transient depressor response from a few mm to 12 mm of Hg. Amounts of pressor substances contained in kidneys also decreased but at a slower rate. They remained near normal levels until the 10th day, then decreased and remained low until the end of the experiment. These results were independent of the method used for determination of pressor substances. It is worth noting that the fall in renal content took place when the rats became acutely hypertensive.

In rats given 1\% NaCl solution as drinking water (group II), blood pressure increased slightly but remained below 150 mm Hg, the upper limit of normal values (fig. 1C). Secretion of pressor substances decreased more gradually than in the preceding groups but did not disappear; residual activity averaging 6 mm Hg was still present on the 22nd day. On the other hand, pressor activity of kidney extracts measured by direct assay or following incubation did not change significantly throughout the experiment.

The effects of DCA in rats given tap water (group III) were intermediate between those obtained in the two preceding groups (fig. 1B). Both renin secretion and content were decreased. Again secretion was affected earlier and more markedly than content. On the 20th day, no pressor activity could be detected in the renal vein effluent while saline extracts of kidneys still gave a pressor response of about 6 mm Hg.

**Discussion**

In the present experiments we have attempted with simple biological procedures to evaluate the pressor activity contained in kidney extracts and released into the renal vein blood and to use such information as an index of renal content and of renal secretion. We are fully aware of the limitations of this type of study and of the greater significance of quantitative determinations of the enzyme renin in kidneys or of blood angiotensin; however, the methods available, while technically more complicated, are not above criticism. Previous results obtained with the procedures used in the present investigation during experimental hypertension and during conditions associated with salt imbalance agree remarkably well with those obtained with more refined and specific methods. There is also evidence that the nephrectomized rat is a satisfactory preparation for the quantitative estimation of renal pressor substances: when pressor responses are plotted against the log-dose of a saline extract of normal kidneys, there is a linear relationship for responses between 15 and 40 mm Hg. A similar curve has been reported following injections of a semi-purified renin preparation. On this basis, we feel justified to assume that changes in the pressor responses to kidney extracts or to renal grafts are an accurate reflection of the amounts of pressor substances present.

These determinations may, however, be vitiated by depressor substance(s). The presence of such substances was more readily seen in some of the kidneys depleted of pressor substances by DCA treatment or by hypertension. They also sometimes occurred in extracts of normal kidneys, where they manifest themselves by an immediate and fleeting fall in pressure, which precedes the usual more delayed and gradual rise in pressure. However, the heights of the pressor responses to such extracts remained within the range of the values obtained from depressor-free extracts, thus suggesting that this depressor activity does not constitute a serious interference.

The present experiments confirm the results of Gross and Sulser on the effects of adrenalectomy and DCA treatment on the
renin content of kidneys and further suggest that increase in content after adrenalectomy and depletion after DCA treatment are respectively associated with hyperactivity and hypoactivity of the renal pressor function. They also indicate that the inhibitory effect of corticosteroids on this function is restricted to those possessing mineralocorticoid activity. Aldosterone, like DCA, depletes kidneys of renin;\textsuperscript{14} cortisone and cortisol have no effect on renin content but cause a reduction in secretion which may be ascribed to the weak sodium-retaining activity of the large doses administered; ACTH which releases gluco- and corticosteroids has no effect on secretion or content. Thus, the present results are consistent with the concept originated years ago\textsuperscript{15} that kidneys and adrenals constitute a close, self-regulatory, and specific endocrine system in which renin is the tropic hormone, the zona glomerulosa the target gland, and a DC-like compound the end effector agent, which in turn, controls renin secretion.

The mode of action of aldosterone or deoxycorticosterone on the juxtaglomerular cells, the probable site of renin formation, may be direct or mediated by the metabolic effects of these steroids. A direct effect, independent of sodium intake, has been ascribed to deoxycorticosterone.\textsuperscript{16} However, there is considerable evidence that sodium retention is of primary importance; addition of salt to a normal diet facilitates the effect of DCA; salt alone in adequate amounts has the same effects as DCA, and sodium deficiency causes hypergranulation of the juxtaglomerular cells in spite of a high aldosterone output.\textsuperscript{17} The way sodium acts on these cells is unknown. A plausible explanation is that besides their endocrine nature, the juxtaglomerular cells act as stretch receptors responsive to volume and pressure.\textsuperscript{2} Thus, the changes in blood volume associated with changes in body sodium and the hypertension caused by sodium retention would supply the necessary stimuli. Our experiments with DCA demonstrate that the metabolic changes brought about by sodium retention are sufficient to inhibit renin secretion completely before hypertension develops. The control of renin secretion by mineralocorticoids is a relatively slow process as compared with the apparent direct and immediate effect of renin or angiotensin on adrenal secretions.\textsuperscript{18,19,20} This is logical, considering that the inhibitory effects of mineralocorticoids represent the feedback mechanism of the renin-aldosterone system.

It has been generally assumed that renin content could be used as an index of the pressor function of kidneys.\textsuperscript{21} While showing that any change in content is accompanied by a change in secretion, the present experiments indicate that a normal renin content does not necessarily reflect a normal secretory activity of the juxtaglomerular cells. Thus a normal content in association with a decreased secretion was found following treatment with cortisone, cortisol, or administration of 1\% saline. Normal content and decrease in secretion was also found during the early phase of treatment with DCA plus salt, or DCA alone. A similar dissociation has been reported following treatment with various renin preparations.\textsuperscript{22}

Dissociation of content and secretion may be explained by the large amounts of pressor substances contained in normal kidneys in contrast to the minute quantities released in the renal vein. Assuming that synthesis and secretion are equally affected, a lack of demand would cause an immediate suppression of synthesis and secretion without influencing, appreciably, renin content. If the lack of demand persists, there would be a gradual atrophy (disuse atrophy) and depletion of the renin-secreting cells. However, if there is only a slight reduction in secretion, changes in the content may be so small percentage-wise that they can remain undetected for a considerable period of time. The same reasoning would apply to situations associated with stimulation of the renal pressor function; an increase in renin content will be secondary to an increase in its release. Thus, while determination of pressor activity in kidney extracts may have some value, determination
of the pressor response to renal grafts appears to be a more sensitive and accurate index of the renal pressor function.

Summary

The pressor response of the nephrectomized rat to the injection of kidney extracts and to a renal graft, was used as an index of the renal content and secretion of pressor substances during adrenal insufficiency or treatment with corticosteroids. Adrenalectomy caused an elevation of both content and secretion, which was best demonstrated after two to three weeks of maintenance therapy. Treatment with cortisol and cortisone decreased slightly but significantly the secretion of pressor substances without altering renal content. ACTH had no effect on either function. Sequential studies of content and secretion of pressor substances during treatment with DCA plus salt showed first a rapid disappearance of pressor activity from renal vein blood followed by a decrease in renal content to near zero levels; DCA without excess salt elicited the same effects but more gradually; administration of 1% saline alone decreased secretion without altering content. It is concluded that salt active corticosteroids specifically inhibit the formation and secretion of renal pressor substances and that determination of the index of secretion constitutes a more reliable and sensitive indicator of the renal pressor function than determination of the pressor activity in kidney extracts.

References


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Circ Res. 1963;12:94-100
doi: 10.1161/01.RES.12.1.94
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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