ALTERATIONS OF ALDOSTERONE BIOSYNTHESIS
BY RAT ADRENAL TISSUE DUE TO INCREASED INTAKE
OF SODIUM AND POTASSIUM

By
Jürg Müller

ABSTRACT

Three groups of rats received respectively the following drinking fluids for two weeks: sucrose 5%; NaCl 0.154 M + sucrose; KCl 0.154 M + sucrose. Aldosterone biosynthesis by quartered adrenal glands of these animals was studied under various in vitro conditions. Adrenals from rats drinking sucrose alone produced significantly more aldosterone under all conditions of incubation than adrenals from rats drinking NaCl, which produced more corticosterone and deoxycorticosterone. Tissue from animals drinking NaCl converted less unlabelled progesterone, 11β-hydroxyprogesterone, deoxycorticosterone and corticosterone to aldosterone and incorporated less tritiated pregnenolone, progesterone, deoxycorticosterone and corticosterone into aldosterone. Adrenals from rats drinking KCl produced less aldosterone than adrenals from rats drinking sucrose under basal conditions but not under stimulation by ACTH or potassium ions. In both groups, the production of corticosterone and of deoxycorticosterone was the same under various test conditions. These results indicate that a high sodium intake inactivates one or both enzymes essential for the conversion of corticosterone to aldosterone, whereas a high potassium intake has no significant effect on these later steps of aldosterone biosynthesis.

Restriction of sodium intake is one of the most potent stimuli of aldosterone secretion in man and experimental animals (Laragh & Kelly 1964; Davis 1967). Similarly a high intake or parenteral administration of potassium salts
lead to increased secretion of aldosterone. The physiological and biochemical mechanisms by which aldosterone secretion is modified in response to alterations in sodium and potassium balance are only partially known, and they still are controversial. The following experiments in intact rats were carried out to study the effects of augmented voluntary sodium and potassium intake on the production of different corticosteroids by isolated adrenal tissue, on the activity of two different aldosterone-stimulating agents added in vitro and on the ability of adrenal tissue to convert various added precursor steroids to aldosterone.

MATERIAL AND METHODS

Animals

Pure-bred male Osborne-Mendel rats weighing between 120 and 150 g were kept for two weeks on rat pellets containing 50 μval of sodium and 187 μval of potassium per gram. The rats were divided into three groups which received respectively the following drinking fluids: Group 1: sucrose 50 g/l; group 2: KCl (0.154 m) + sucrose; group 3: NaCl (0.154 m) + sucrose. As has been described by Hall & Hall (1964) the voluntary fluid consumption was considerably increased when sucrose was added to the salt solutions. The average daily consumption per rat was 60 ml of sucrose, 48 ml of KCl + sucrose and 56 ml of NaCl + sucrose. All groups of rats appeared to be in good health at the end of two weeks and did not differ in body or adrenal weight.

Incubation Procedure

According to a previously described procedure (Müller 1965), eight homogeneous pools of quartered adrenal glands from twelve rats were used for each assay. After preincubation for 30 minutes in a modified Krebs-Ringer bicarbonate buffer containing 3.63 mval of potassium and 2 g of glucose per litre, the final incubations were carried out for two hours in 6 ml of buffer to which the following substances were added: KCl (final K+ concentration 8.5 mval/l), ACTH (Cortrophine®, Organon, 5 IU per flask), unlabelled steroids (250 μg in 0.05 ml of ethanol per flask) or trace amounts of tritium-labelled steroids (in 0.05 ml of ethanol per flask).

Steroid Assays

Final concentrations of aldosterone, corticosterone and deoxycorticosterone in the incubation medium were determined by previously described and evaluated modifications (Müller 1965; Müller & Weick 1967) of the double isotope dilution derivative assay by Kliman & Peterson (1960) and Peterson (1960). Tritium-labelled aldosterone was determined by a double isotope dilution procedure (Müller 1966). Porter-Silber chromogens were determined by the method of Peterson et al. (1957). Several investigators have found that the main Porter-Silber chromogen secreted by the rat adrenal gland is 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) (Birmingham & Ward 1961; Cortés et al. 1963), whereas little if any cortisol is secreted in this animal
species (Bush 1953). However, since no pure 18-OH-DOC was available, cortisol was used as the standard substance, and Porter-Silber chromogens are expressed as µg of cortisol equivalents.

RESULTS

Aldosterone Production

Baseline aldosterone production was 75% higher in adrenals of rats drinking sucrose alone than in the tissue of rats receiving either KCl or NaCl (Table 1). On the other hand, when KCl, ACTH or progesterone was added to the incubation medium, there was no significant difference between the aldosterone production of adrenals from rats drinking sucrose or KCl. In the tissue of rats on NaCl, stimulated aldosterone production was 50% lower. In each group, the stimulation was somewhat greater with ACTH than with a high potassium concentration, but the differences were not significant. In all three groups, addition of progesterone to the incubation medium stimulated aldosterone production to the same extent as ACTH.

Corticosterone Production

At the low and at the high potassium concentration, corticosterone production was approximately twice as high in the adrenals of rats drinking NaCl than in the adrenals of the other two groups of animals (Table 2). When ACTH or progesterone was added to the incubation medium, corticosterone production was the same in all three groups.

Table 1.

Aldosterone production in vitro by adrenal tissue of rats receiving different drinking fluids for two weeks. Mean values of two assays (µg/100 mg · 2 h ± standard deviation, N = 4). P values of significance were calculated by t tests and refer to differences between adjacent lines and columns, respectively.

<table>
<thead>
<tr>
<th>Additions to incubation medium</th>
<th>Sucrose</th>
<th>Drinking fluid KCl + sucrose</th>
<th>NaCl + sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>3.68 ± 0.84</td>
<td>2.14 ± 0.24</td>
<td>1.95 ± 0.64</td>
</tr>
<tr>
<td>KCl*</td>
<td>8.14 ± 0.40</td>
<td>7.15 ± 0.88</td>
<td>4.11 ± 0.26</td>
</tr>
<tr>
<td>ACTH**</td>
<td>9.71 ± 2.43</td>
<td>9.80 ± 1.46</td>
<td>4.75 ± 0.77</td>
</tr>
<tr>
<td>Progesterone***</td>
<td>11.73 ± 1.09</td>
<td>12.20 ± 1.82</td>
<td>4.51 ± 0.35</td>
</tr>
</tbody>
</table>

* 8.5 mval K⁺/l  ** 5 IU per flask  *** 250 µg per flask

Downloaded from Bioscientifica.com at 11/10/2018 02:16:42AM via free access
Table 2.
Corticosterone production in vitro. Mean values of two assays
(\(\mu g/100 \text{ mg} \cdot 2 \text{ h} \pm \text{ standard deviation, } N = 4\)).

<table>
<thead>
<tr>
<th>Additions to incubation medium</th>
<th>Sucrose</th>
<th>Drinking fluid</th>
<th>NaCl + sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KCl + sucrose</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>8.01 ± 1.99</td>
<td>((P &gt; 0.4)) 6.96 ± 0.51</td>
<td>((P &lt; .001)) 14.3 ± 4.6</td>
</tr>
<tr>
<td>ACTH</td>
<td>48.0 ± 6.4</td>
<td>((P &gt; 0.3)) 52.5 ± 2.9</td>
<td>((P &gt; 0.6)) 54.6 ± 6.4</td>
</tr>
<tr>
<td>Progesterone</td>
<td>64.6 ± 18.5</td>
<td>((P &gt; 0.8)) 67.4 ± 14.8</td>
<td>((P &gt; 0.7)) 64.7 ± 9.2</td>
</tr>
</tbody>
</table>

Deoxycorticosterone Production

Production of deoxycorticosterone was the same under all test conditions in the adrenals of rats on sucrose and in the adrenals of rats given KCl. It was significantly higher in the adrenals of rats drinking NaCl with or without KCl or ACTH (Table 3). In this latter group, addition of KCl to the incubation medium stimulated deoxycorticosterone production by 400%, but only by 200% in the other two groups.

Production of Porter-Silber Chromogens

Similar amounts of Porter-Silber chromogens (»18-OH-DOC«) were produced by adrenal tissue from all three groups of animals with or without KCl, ACTH or progesterone (Table 4). While the addition of ACTH or pro-

Table 3.
Deoxycorticosterone production in vitro. Mean values of two assays
(\(\mu g/100 \text{ mg} \cdot 2 \text{ h} \pm \text{ standard deviation, } N = 4\)).

<table>
<thead>
<tr>
<th>Additions to incubation medium</th>
<th>Sucrose</th>
<th>Drinking fluid</th>
<th>NaCl + sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KCl + sucrose</td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>0.52 ± 0.02</td>
<td>((P &lt; .001)) 0.57 ± 0.31</td>
<td>((P &lt; .001)) 1.60 ± 0.45</td>
</tr>
<tr>
<td>ACTH</td>
<td>1.84 ± 0.21</td>
<td>((P &lt; .001)) 2.16 ± 0.49</td>
<td>((P &lt; .001)) 3.90 ± 0.55</td>
</tr>
<tr>
<td>Progesterone</td>
<td>39.4 ± 6.5</td>
<td>((P &gt; 0.2)) 32.6 ± 6.0</td>
<td>((P &gt; 0.8)) 31.7 ± 3.6</td>
</tr>
</tbody>
</table>
Table 4.
Production of Porter-Silber chromogen («18-OH-DOC») in vitro. Mean values of two assays (µg/100 mg · 2 h ± standard deviation, N = 4).

<table>
<thead>
<tr>
<th>Additions to incubation medium</th>
<th>Drinking fluid</th>
<th>NaCl + sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KCl + sucrose</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>6.9 ± 0.3</td>
<td>11.4 ± 5.7</td>
</tr>
<tr>
<td>(P &gt; 0.2)</td>
<td>(P &lt; 0.005)</td>
<td>(P &gt; 0.5)</td>
</tr>
<tr>
<td>KCl</td>
<td>10.4 ± 1.0</td>
<td>14.5 ± 5.0</td>
</tr>
<tr>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>ACTH</td>
<td>52.2 ± 6.6</td>
<td>57.2 ± 11.1</td>
</tr>
<tr>
<td>(P &gt; 0.25)</td>
<td>(P &gt; 0.5)</td>
<td>(P &gt; 0.7)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>64.8 ± 17.0</td>
<td>60.5 ± 11.6</td>
</tr>
<tr>
<td>(P &gt; 0.3)</td>
<td>(P &gt; 0.8)</td>
<td></td>
</tr>
</tbody>
</table>

Additions to incubation medium

--
(7.5 ± 0.8) (P > 0.25)
(9.1 ± 1.8) (P > 0.25)
(38.1 ± 5.5) (P < 0.05)
(51.5 ± 12.8) (P > 0.3)

KCl
(9.1 ± 1.8) (P > 0.25)
(10.4 ± 1.0) (P > 0.2)
(52.2 ± 6.6) (P > 0.5)
(64.8 ± 17.0) (P > 0.8)

AC TH
(38.1 ± 5.5) (P < 0.05)
(52.2 ± 6.6) (P > 0.5)
(57.2 ± 11.1) (P > 0.7)

Progesterone
(51.5 ± 12.8) (P > 0.3)
(64.8 ± 17.0) (P > 0.8)

The incorporation of all four tritiated precursors into aldosterone was uniformly reduced by about 60% in the adrenals of rats drinking NaCl.

Adrenal tissue of animals drinking sucrose converted more tritiated pregnenolone, progesterone, deoxycorticosterone and corticosterone to aldosterone than the tissues of animals drinking either of the salt solutions (Table 6). Adrenals of rats drinking KCl incorporated tritiated corticosterone into aldosterone almost to the same extent as adrenals of rats on sucrose, but the conversion of the other three radioactive substrates was reduced by 26%. The incorporation of all four tritiated precursors into aldosterone was uniformly reduced by about 60% in the adrenals of rats drinking NaCl.
Table 5.
Steroid production *in vitro*. Mean values of two flasks of one assay (μg/100 mg • 2 h ± range).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Aldosterone production</th>
<th>Porter-Silber chromogen production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucrose</td>
<td>Drinking fluid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>4.16 ± 0.36</td>
<td>2.31 ± 0.25</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>14.43 ± 2.67</td>
<td>12.25 ± 3.44</td>
</tr>
<tr>
<td>11β-hydroxyprogesterone</td>
<td>18.02 ± 0.10</td>
<td>13.68 ± 0.57</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>19.79 ± 1.92</td>
<td>16.02 ± 0.59</td>
</tr>
</tbody>
</table>
Table 6.
Conversion of tritiated precursors to aldosterone-[\(^{3}\text{H}\)]; cpm/100 mg × 10\(^{-3}\).
Mean values of two flasks ± range.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Sucrose</th>
<th>Drinking fluid</th>
<th>NaCl + sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnenolone-[(^{7}\alpha-\text{H})]</td>
<td>116.7 ± 6.3</td>
<td>89.0 ± 9.1</td>
<td>50.1 ± 3.3</td>
</tr>
<tr>
<td>(2.06 × 10(^{6}) cpm per 100 mg tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone-[(^{7}\alpha-\text{H})]</td>
<td>148.8 ± 9.8</td>
<td>110.7 ± 6.0</td>
<td>63.6 ± 9.7</td>
</tr>
<tr>
<td>(2.36 × 10(^{6}) cpm per 100 mg tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxycorticosterone-[(^{1,2}-\text{H})]</td>
<td>76.9 ± 0.3</td>
<td>55.7 ± 5.1</td>
<td>32.5 ± 2.4</td>
</tr>
<tr>
<td>(1.55 × 10(^{6}) cpm per 100 mg tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosterone-[(^{1,2}-\text{H})]</td>
<td>67.8 ± 4.8</td>
<td>63.2 ± 3.4</td>
<td>25.8 ± 2.9</td>
</tr>
<tr>
<td>(2.26 × 10(^{6}) cpm per 100 mg tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Recent studies on the site of action of sodium depletion on aldosterone biosynthesis have led to apparently contradictory results. Bledsoe et al. (1966) found indirect evidence that aldosterone biosynthesis in man was stimulated by sodium depletion at a step preceding the formation of deoxycorticosterone. This was consistent with *in vitro* studies, which indicated that angiotensin II did not stimulate the conversion of progesterone, deoxycorticosterone or corticosterone to aldosterone but acted rather on the conversion of cholesterol to pregnenolone (Kaplan & Bartter 1962; Müller 1966) or even at steps preceding the formation of cholesterol (Lommer & Wolff 1966). Angiotensin II is generally considered to be the most important physiological mediator of an increased aldosterone secretion in sodium deficiency (Binnion et al. 1965). On the other hand, adrenal tissue from sodium-deficient dogs converted more progesterone and corticosterone to aldosterone than the adrenal tissue of sodium-repleted dogs (Dawis et al. 1966). More corticosterone was converted to aldosterone by adrenal mitochondria from sodium-deficient rats than by mitochondria from control animals (Marusic & Mulrow 1967). The results of the present studies confirm that later steps in aldosterone biosynthesis are significantly modified by chronic changes in sodium intake. They are also compatible with the view that aldosterone-stimulating substances do not act on the enzymes converting corticosterone to aldosterone during short-time incubations, but rather that they increase the amount of substrate for these reactions (Müller 1966). Thus ACTH or potassium ions stimulated aldosterone production in each pool of adrenal tissue only to the level obtained when the substrate, i.e. progesterone, was added to the incubation medium.
Addition of NaCl to the drinking fluid of rats feeding on pellets with a moderate sodium content led to the following manifestations of altered aldosterone biosynthesis: decreased production of aldosterone both with and without stimulating agents; increased production of two biological precursors of aldosterone, i.e. corticosterone and deoxycorticosterone; decreased conversion of unlabelled progesterone, 11β-hydroxyprogesterone, deoxycorticosterone and corticosterone to aldosterone; decreased incorporation of tritiated pregnenolone, progesterone, deoxycorticosterone and corticosterone into aldosterone. Reduced activity of one or both of the enzymes necessary for the conversion of corticosterone to aldosterone, i.e. 18-hydroxylase or 18-hydroxydehydrogenase, could account for all these findings.

Ulick et al. (1964) have shown that the secretion rates of aldosterone and of 18-hydroxycorticosterone in man are equally increased during sodium-deficiency. But in vitro studies by Vecsei et al. (1966) indicated that in the adrenal tissue of rats drinking NaCl the conversion of tritiated progesterone to 18-hydroxycorticosterone was less decreased than the conversion of tritiated progesterone to aldosterone. In the present studies 18-hydroxycorticosterone was not measured, but the production of 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) was estimated by the Porter-Silber reaction. We could find no difference in the production of Porter-Silber chromogens by the adrenal tissue of rats receiving different drinking fluids. Addition of KCl to the incubation medium stimulated the production of Porter-Silber chromogens only to about the same extent as corticosterone production. Since 18-OH-DOC — in contrast to 18-hydroxycorticosterone — is mainly produced in the zona fasciculata/reticularis (Sheppard et al. 1963; Stachenko et al. 1964), it may not be a natural precursor of aldosterone, which originates from the zona glomerulosa. In addition, the Porter-Silber reaction may not be a reliable index of 18-OH-DOC production. Whereas 18-OH-DOC has been said to be the only steroid secreted by the rat adrenal gland giving a positive Porter-Silber reaction (Birmingham & Ward 1961; Cortés et al. 1963), the results in Table 5 clearly show that rat adrenal tissue can form substantial amounts of Porter-Silber chromogens from 11β-hydroxysteroids.

Deoxycorticosterone, which is produced mainly in the zona glomerulosa (Sheppard et al. 1963), was stimulated up to 400% above control values when KCl was added to the incubation medium. In the adrenal tissue of rats drinking NaCl, deoxycorticosterone production was strikingly increased with or without KCl or ACTH. This finding was suggestive of an inhibition of aldosterone biosynthesis at the level of 11β-hydroxylation. In the adrenal tissue of rats drinking NaCl, however, the conversion of 11β-hydroxylated steroids to aldosterone was decreased to the same extent as the conversion of 11-deoxysteroids. Moreover, the absolute increase of corticosterone production at the low as well as at the high potassium concentration in the adrenals of rats drinking NaCl.
was greater than the absolute increase of deoxycorticosterone production. The smaller relative increase of corticosterone production is probably due to the fact that most of the corticosterone is produced by the zona fasciculata/reticularis.

While addition of NaCl to the drinking fluid led to marked alterations of aldosterone biosynthesis, KCl was in most respects ineffective. Thus, adrenals of rats drinking KCl produced the same amount of aldosterone as the adrenals of rats drinking sucrose, when they were stimulated by potassium ions or by ACTH. Production of corticosterone and of deoxycorticosterone, respectively, was the same in tissue from both groups of rats under all experimental conditions. Conversion of several unlabelled and tritiated precursor steroids to aldosterone was slightly lower in the tissues of rats drinking KCl than in the tissue of rats drinking sucrose. Moreover, the base-line aldosterone production was as low in the adrenals of rats drinking KCl as in the adrenals of rats drinking NaCl. We cannot explain this finding. However, it demonstrates that the aldosterone production by adrenal tissue incubated in the absence of exogenous precursor steroids or stimulating agents is not a reliable index of the adrenal's capacity to produce aldosterone. Potassium ions directly stimulate aldosterone secretion in vivo (Blair-West et al. 1963; Davis et al. 1963) and aldosterone biosynthesis in vitro (Giroud et al. 1956; Müller 1965; Kaplan 1965) probably by stimulating the conversion of cholesterol to pregnenolone in zona glomerulosa cells (Müller 1966). But the chronic changes in the later steps of aldosterone biosynthesis observed during alteration of sodium intake seem to be relatively independent of the potassium intake. The different site of action on aldosterone biosynthesis by sodium and potassium could explain the additive effect of potassium administration on the aldosterone secretion.

\[ \text{Cholesterol} \leftarrow \text{Angiotensin II} \leftarrow \text{sodium depletion} \]
\[ \text{Pregnenolone} \]
\[ \text{Progesterone} \]
\[ \text{Deoxycorticosterone} \]
\[ \text{Corticosterone} \leftarrow \text{sodium depletion} \]
\[ \text{18-Hydroxycorticosterone} \]
\[ \text{Aldosterone} \]

**Fig. 1.**
Effects of sodium depletion on aldosterone biosynthesis.
\[ \leftarrow \text{direct stimulation.} \quad \leftarrow \text{indirect stimulation.} \]

35
rate of sodium depleted patients (Cannon et al. 1966). The greater responses of aldosterone secretion to ACTH administration that have been observed in sodium-deficient men (Muller 1962) and dogs (Ganong et al. 1966) could be similarly explained.

As shown in Fig. 1, two different biochemical mechanisms may account for the increased aldosterone secretion in response to sodium depletion; activation of early steps of aldosterone biosynthesis by increased amounts of circulating angiotensin II and activation of one or both of the enzymes necessary for the conversion of corticosterone to aldosterone. While it has been demonstrated that sodium deficiency leads to increased levels of renin (Gross 1964) and of angiotensin II (Scornik & Paladini 1964) and that angiotensin II directly stimulates aldosterone secretion by the adrenal cortex (Davis 1961), the mechanism by which changes in sodium intake can mediate changes in the activity of 18-hydroxylase or 18-hydroxydehydrogenase is as yet unknown.

ACKNOWLEDGEMENTS

The author is most grateful to Mrs. G. Möhren and Mrs. D. Vogelsang for their excellent technical assistance and to Dr. E. R. Froesch and Prof. A. Labhart for valuable suggestions, criticism and encouragement.

This work was supported by Grant 3853 from the Schweizerische Nationalfonds. Unlabelled steroids used in this study were a gift from Ciba AG, Basel.

REFERENCES

Davis J. O.: Recent Progr. Hormone Res. 17 (1961) 293.

Received on July 26th, 1967.