THE EFFECT OF OUABAIN ON ALDOSTERONE PRODUCTION IN THE RAT

By
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ABSTRACT

Chronic treatment with ouabain increases aldosterone production in the rat but has no effect on the renal renin content. The effect of ouabain in vitro on aldosterone depends on its concentration and the K content of the media: \(10^{-8}\) M ouabain and 4 and 8 meq./l K and \(10^{-4}\) M and 4 meq./l K intensify, while \(10^{-3}\) M at 6 and 8 meq./l K inhibits production. In the present experiments, ouabain at low concentration increased and at high concentration reduced aldosterone production.

In addition to ACTH and angiotensin, extracellular concentrations of potassium above and of sodium below normal are known to stimulate aldosterone secretion (for review see Davis 1967).

In a recent study (Szalay 1969) it was shown that angiotensin inhibits K accumulation in the adrenal cortex. With this in mind, we assumed that angiotensin interferes with the work of the Na-pump, thereby increasing the K and reducing the Na concentration in the extracellular space and so brings about an ionic milieu that promotes aldosterone secretion. We further inferred that if our assumption proved correct then other compounds which inhibit the Na-pump would similarly stimulate the production of aldosterone. With the knowledge that ouabain is such an inhibitor of specific action we studied the effect of chronic administration of ouabain as well as ouabain in vitro, for the effects it exerts on aldosterone production.
MATERIALS AND METHODS

Chronic treatment with ouabain in vivo

Three groups of CFE rats weighing 190 ± 10 g and maintained at 23°C on a standard diet were given respectively a subcutaneous injection of 0.38, 0.75 and 1.5 mg/kg b.w. of ouabain daily for 14 days. The rats of the control groups each received 0.5 ml of physiological saline daily for the same period and by the same route. Twenty-four hours after the last injection the animals under slight ether anaesthesia were exsanguinated through a cannula inserted into the aorta, and the adrenals and kidneys were removed. The adrenals of 3 rats at a time were quartered and incubated for 120 min at 37°C in 5 ml of a Krebs-Ringer bicarbonate buffer, pH 7.4, containing 200 mg glucose per 100 ml under an atmosphere of 95% oxygen and 5% carbon dioxide. The aldosterone and corticosterone contents of the medium were then determined. The rate of aldosterone secretion of the adrenals in vitro was used as an index of aldosterone secretion in vivo (van der Wal et al. 1965).

The kidney renin content was determined by the indirect method of Gross et al. (1965), and the plasma corticosterone concentration by spectrofluorimetry according to Guillenin et al. (1959). The serum K and Na contents were measured by flame photometry.

Ouabain in vitro

The adrenals of female rats weighing 200 ± 20 g were removed, cleaned, placed on filter paper moistened with physiological saline and kept on ice. The experiments were carried out in randomized blocks. The adrenals were quartered and the quarters within each block were evenly distributed so as to have 20 of the adrenal-quarters in each flask (after wet weight determination). The flasks were preincubated for 40 min in KRB-glucose buffer containing 4 meq. of K, after which the medium was discarded and the effect on aldosterone of various concentrations of ouabain in KRB-glucose buffer with different K contents, was determined during incubation for 120 min.

The experiments were performed under each of the following conditions:

10⁻⁸ M/l ouabain and 4 and 8 meq./K
10⁻⁸ M/l ouabain and 6 meq./K
10⁻⁴ M/l ouabain and 4, 6 and 8 meq./l K
10⁻³ M/l ouabain and 4, 6 and 8 meq./l K

Control tubes were incubated without ouabain in buffers of the same K concentration as for the actual experiments.

Corticosteroid determinations

The adrenocortical hormones were extracted from the medium with chloroform, purified by Bondy’s method (Bondy & Upton 1957) and isolated by paper chromatography in the Bush B₃ system (Bush 1952). The paper was then chromatographed once more for 16 hours in the system used for purification in order to intensify the tetrazolium blue reaction (Kemény et al. 1962). After the reaction the formazan spots were cut out at the places corresponding to the aldosterone and corticosterone standards, eluted with a 7:3 mixture of ethylacetate and methanol, and measured in a Unicam spectrophotometer at 520 nm wavelength.

After incubation both in the in vivo and in the in vitro experiments we determined the wet and the dry weights of the adrenal slices. The tissues were then subjected to
digestion in 0.1 N HNO₃, then filtered and diluted after which the K content was determined in a flame photometer.

In the in vivo experiment analysis of variance was performed according to Dunnet's test (1955), while in the in vitro experiment, a three-way analysis of variance was done. In the latter case block x treatment interactions were used as error variance, and further analysis was performed using a multiple comparison test with a significance level of 0.1 (Scheffé 1959).

**RESULTS**

*In vivo experiment* (Table 1)

Chronic treatment with 0.75 mg/kg b.w. of ouabain increased aldosterone production (*P* < 0.01). No significant effects were obtained with a dose of 0.38 and 1.5 mg. Ouabain caused no change in corticosterone production or plasma corticosterone concentration. The two larger doses caused an increase in adrenal weight.

Treatment with a dose of 0.75 mg ouabain increased the serum Na concentration, but not significantly, and had no effect on the K concentration. The K content of the adrenal tissue and the renin content of the kidney remained unchanged.

*In vitro experiment*

In all series of experiments the medium containing 6 and 8 meq./l K produced a significantly higher rate of aldosterone and corticosterone secretion than the medium containing 4 meq./l K (*P* < 0.05; Fig. 1).

10⁻⁵ M ouabain increased aldosterone production significantly at 4 and 8 meq./l K (*P* < 0.01), and corticosterone production at 8 meq./l K (*P* < 0.05; Fig. 1. a₁, a₂).

10⁻⁸ M ouabain at 6 meq./l K had no effect on corticosteroid production (Table 2).

An interaction was found between the effect of 10⁻¹ M ouabain and of K both on aldosterone and corticosterone production (*P* < 0.025). The 4 meq./l of K × ouabain interaction was + 0.4 µg for aldosterone (*P* < 0.1) and + 0.5 µg for corticosterone (*P* < 0.01). The 8 meq. of K × ouabain interaction for aldosterone was −0.3 µg. The combined effect of 8 meq./l of K × ouabain and 4 meq./l of K × ouabain was significant for aldosterone (*P* < 0.025). The 8 meq./l of K × ouabain interaction for corticosterone was −0.3 µg (*P* < 0.1; Fig. 1. b₁, b₂).

10⁻³ M ouabain inhibited aldosterone production at 6 and 8 meq./l K (*P* < 0.01); at 4 meq./l K it was ineffective, and at no K concentration did it affect corticosterone production (Fig. 1, c₁, c₂).

The adrenal K content was unaffected by 10⁻⁸ M ouabain, and significantly decreased by 10⁻⁴ M and 10⁻³ M ouabain regardless of the K concentration of the media (Table 3).
<table>
<thead>
<tr>
<th></th>
<th>14 daily sc phys. sal. inj.</th>
<th>14 daily sc ouabain injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal weight mg</td>
<td>54.6 ± 1.3*(26)</td>
<td>55.0 ± 2.1 (15)</td>
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<tr>
<td></td>
<td></td>
<td>60.0 ± 1.5b(30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68.0 ± 2.0a(15)</td>
</tr>
<tr>
<td>Aldosterone production µg/100 mg adrenal/2 h</td>
<td>1.3 ± 0.3 (8)</td>
<td>2.4 ± 0.4c(5)</td>
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<tr>
<td></td>
<td></td>
<td>2.6 ± 0.3a(9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.9 ± 0.3 (5)</td>
</tr>
<tr>
<td>Corticosterone production µg/100 mg adrenal/2 h</td>
<td>6.6 ± 1.2 (8)</td>
<td>8.4 ± 1.7 (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8 ± 0.9 (9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.5 ± 0.5 (5)</td>
</tr>
<tr>
<td>Plasma corticosterone µg/100 ml</td>
<td>22.2 ± 2.1 (26)</td>
<td>19.9 ± 2.3 (15)</td>
</tr>
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<td></td>
<td></td>
<td>19.5 ± 2.4 (28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.3 ± 3.8 (15)</td>
</tr>
<tr>
<td>Adrenal K content meq./kg dry weight</td>
<td>128.3 ± 4.9 (8)</td>
<td>155.1 ± 5.3 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>129.7 ± 7.3 (10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>141.5 ± 6.2 (4)</td>
</tr>
<tr>
<td>Serum K meq./l</td>
<td>4.4 ± 0.3 (15)</td>
<td>4.5 ± 0.4 (15)</td>
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<tr>
<td></td>
<td></td>
<td>4.6 ± 0.7 (15)</td>
</tr>
<tr>
<td>Serum Na meq./l</td>
<td>129.5 ± 2.1 (15)</td>
<td>129.0 ± 1.1 (15)</td>
</tr>
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<td></td>
<td></td>
<td>137.1 ± 1.6 (15)</td>
</tr>
<tr>
<td>Renal renin content angiotensin µg/g kidney</td>
<td>82.7 ± 12.1(10)</td>
<td>74.7 ± 7.0 (15)</td>
</tr>
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<td></td>
<td></td>
<td>72.0 ± 5.6 (16)</td>
</tr>
</tbody>
</table>

*: mean ± se
( ): number of determinations
a: as against control P < 0.01
b: as against control P < 0.05
c: as against control P > 0.05
Fig. 1.
Effect of different concentrations of ouabain and 4, 6 and 8 meq./l K on the in vitro production of aldosterone and corticosterone by rat adrenals (mean ± se).
Unbroken line: controls.

Table 2.
Effect of 10⁻⁸ M ouabain at 6 meq./l K on the in vitro production of aldosterone and corticosterone.

<table>
<thead>
<tr>
<th>Control</th>
<th>10⁻⁸ M ouabain at 6 meq./l K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td>Corticosterone</td>
</tr>
<tr>
<td>1.9 ± 0.3*</td>
<td>3.5 ± 0.3</td>
</tr>
</tbody>
</table>

*: mean ± se. Values are in µg/100 mg adrenal
Number of determinations: 13

481
Table 3.
Adrenal K content in meq./kg dry weight.

<table>
<thead>
<tr>
<th>Concentration of ouabain</th>
<th>4 meq./l K</th>
<th></th>
<th>6 meq./l K</th>
<th></th>
<th>8 meq./l K</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ouabain</td>
<td>Control</td>
<td>Ouabain</td>
<td>Control</td>
<td>Ouabain</td>
</tr>
<tr>
<td>$10^{-8}$ M (19)</td>
<td>143.5 ± 9.2$^a$</td>
<td>151.6 ± 7.4$^a$</td>
<td>no determination</td>
<td></td>
<td>153.9 ± 5.9</td>
<td>167.5 ± 8.5$^a$</td>
</tr>
<tr>
<td>$10^{-4}$ M (14)</td>
<td>117.6 ± 5.2</td>
<td>98.4 ± 4.6$^a$</td>
<td>130.8 ± 5.3</td>
<td>111.5 ± 5.1$^a$</td>
<td>132.6 ± 8.8</td>
<td>118.2 ± 7.6$^a$</td>
</tr>
<tr>
<td>$10^{-3}$ M (14)</td>
<td>112.1 ± 9.4</td>
<td>56.0 ± 3.1$^a$</td>
<td>101.6 ± 4.9</td>
<td>61.0 ± 5.1$^a$</td>
<td>103.9 ± 8.7</td>
<td>72.6 ± 5.7$^a$</td>
</tr>
</tbody>
</table>

*: mean ± se
a: as against control $P < 0.01$
( ): number of determinations
DISCUSSION

In the present experiments, prolonged treatment of rats with ouabain increased the production of aldosterone, but this increase was not brought about by the renin-angiotensin system. Nor does it seem likely that the production was intensified by ACTH secretion, since in our hands, ouabain influenced neither the in vitro corticosterone secretion nor the plasma corticosterone level. It is possible that in augmenting aldosterone production ouabain acts directly on the adrenal gland: Metzler & Greeff (1954) found that prolonged ouabain administration causes adrenal hypertrophy in the hypophysectomized rat.

Deane & Gardner (1951) and Gardner et al. (1954) reported widening of the zona glomerulosa in the rat after prolonged treatment with ouabain. Several investigators observed adrenal hypertrophy following ouabain or k-strophantin administration (Pyörälä & Eränkö 1957; Gomoll 1967).

The present work shows that at a given K concentration certain ouabain concentrations have a stimulating effect; for instance, at $10^{-8}$ m ouabain and 4 and 8 meq./l K and $10^{-4}$ m and 4 meq./l K increase the production whereas higher ouabain concentrations at higher K concentrations inhibit it. The effect of ouabain on aldosterone production offers no parallel to the action it exerts on the K content of adrenal tissue: $10^{-8}$ m ouabain did not influence the K content of tissue but increased the production of aldosterone; $10^{-4}$ m ouabain reduced the tissue K content at every K concentration, but intensified aldosterone production only at 4 meq./l K; lastly, $10^{-3}$ m ouabain similarly reduced adrenal K content and the production of aldosterone.

In our view, ouabain augments aldosterone production at a concentration ($10^{-8}$ m) i.e. a concentration which still slightly inhibits Na-K-ATPase. This slight inhibition seems sufficient to increase the ratio of extracellular to intracellular K and of intracellular to extracellular Na in the zona glomerulosa, and the resulting ionic milieu stimulates aldosterone production. A high concentration of ouabain ($10^{-3}$ m) inhibits the active transport and cellular metabolism, and this is the reason for the decrease in aldosterone production.

Cushman (1969) found that $5.5 \times 10^{-5}$ m ouabain reduced aldosterone production by adrenal-cortical slices of the dog in media containing 5.8 or 9.8 meq./l K. Wellen & Benraad (1969) observed that $10^{-7}$ m ouabain was sufficient to reduce corticosterone production by the adrenals of the calf. These data are not inconsistent with our present ones, as the rat is known to be highly insensitive to ouabain; Akera et al. (1969) showed that to attain a 50% inhibition of cardiac Na-K-ATPase, 40 to 100 times as much ouabain is required for the rat as for other animal species.

Of interest is the fact that, in our experiments ouabain had less effect on corticosterone than on aldosterone production. In work done by Cushman (1969) $5.5 \times 10^{-8}$ m ouabain failed to reduce corticosterone and 17-OH-corti-
coid production. It may be that ouabain acts specifically to some extent on the zona glomerulosa. Autoradiographic studies of Bretschneider et al. (1962) give support to this suggestion in that another digitalis glycoside, 14C-labelled Lanatosid-C, has been shown to be bound specifically to the adrenal cortex, particularly to the zona glomerulosa.

We are unable to explain why $10^{-4}$ M ouabain reduces aldosterone production at 8 meq./l K and why $10^{-8}$ M and $10^{-4}$ M is ineffective at 6 meq./l K. Apart from inhibiting Na-K-ATPase ouabain may have an as yet unknown effect by which it influences the production of aldosterone.

REFERENCES


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