EFFECTS OF PROLONGED INFUSIONS OF
POTASSIUM CHLORIDE, ADRENOCORTICOTROPHIN
OR ANGIOTENSIN II UPON
SERUM ALDOSTERONE CONCENTRATION
AND THE CONVERSION OF CORTICOSTERONE
TO ALDOSTERONE IN RATS

By
Jan Komor and Jürg Müller

ABSTRACT

The temporal relation between alterations in serum aldosterone and in the conversion of labelled corticosterone to aldosterone by incubated adrenal tissue was studied in conscious rats receiving long-term infusions of KCl, ACTH or angiotensin II. When potassium-deficient rats were given KCl, a marked increase in serum aldosterone was observed only after 12 h, i.e. at a time when the conversion of corticosterone to aldosterone had become normal. After 24 h of ACTH infusion into sodium- and potassium-replete rats the serum aldosterone was markedly elevated, whereas the conversion of corticosterone to aldosterone was significantly decreased. After 48 h of continued ACTH infusion the serum aldosterone returned to normal and there was a further decrease in the conversion rate. A 24-h angiotensin II infusion into sodium- and potassium-replete rats induced significant increases in both the serum aldosterone and the conversion. After 48 h of continued angiotensin infusion the serum aldosterone returned to normal while the conversion and the blood pressure remained elevated. These results indicate that the activity of the enzymes involved in the final steps of aldosterone biosynthesis may become rate-limiting for the secretion of aldosterone during potassium deficiency and during prolonged ACTH treatment. On the other hand, the observed transiency of aldosterone stimulation by exogenous angiotensin II was not due to a suppression of the final steps of aldosterone biosynthesis and remains unexplained.

Address reprint requests to Prof. J. Müller, Steroidlabor, Universitätsspital, CH-8091 Zürich, Switzerland.
Alterations in the activity of one or more of the enzymes involved in late steps of aldosterone biosynthesis can be induced in rats or dogs by changes in sodium or potassium balance and may play an important role in the multifactorial control system which adapts the secretion of aldosterone to the needs of the organism (Vecsei et al. 1966a; Marusic & Mulrow 1967; Davis et al. 1968; Müller & Huber 1969; Boyd et al. 1971; Baumann & Müller 1972a,b; Aguilera et al. 1975). In particular, they could be involved in negative feedback regulation, since a marked decrease in the conversion of deoxycorticosterone and corticosterone to aldosterone can be observed not only after sodium loading or potassium restriction but also after treatment of rats with exogenous mineralocorticoids (Hunziker & Müller 1977). Such a feedback suppression of late steps of aldosterone biosynthesis might explain why ACTH in man (Müller et al. 1956) and angiotensin II in sheep (Blair-West et al. 1962) and dogs (McCaa et al. 1975) have only a transient stimulatory effect upon aldosterone secretion, because these stimulators have been found to act primarily on an early biosynthetic step (Müller 1966). The following experiments were undertaken in order to study changes in late steps of aldosterone biosynthesis in the course of prolonged infusions of KCl, ACTH or angiotensin II into intact conscious rats. By simultaneously measuring circulating aldosterone levels and the adrenals' capacity to convert corticosterone to aldosterone in individual rats we hoped to elucidate more clearly the contribution of changes in late biosynthetic steps to the long-term alterations of aldosterone secretion in response to these stimulators.

**MATERIALS AND METHODS**

**Rats and diets**

Pure-bred male Osborne-Mendel rats (Zb: CARA) weighing between 200 and 220 g were studied. Prior to the infusion experiments, they were kept for two weeks on one of the following semisynthetic diets: "complete": 230 mmol Na+ and 230 mmol K+ per kg, "potassium-deficient": 230 mmol Na+ and 0.7 mmol K+ per kg. With minor modifications, these diets were made up according to Hartroft & Eisenstein (1957). During infusion with angiotensin II, the rats were given a sodium- and potassium-deficient diet (5 mmol Na+ and 0.7 mmol K+ per kg) but received NaCl (2.5 mmol/day) and KCl (2.4 mmol/day) in the infusion fluid.

**Cannulation and infusion**

Under ether anaesthesia, a catheter (Silastic, medical grade tubing, outer diameter 0.94 mm, internal diameter 0.51 mm) was entered through a small incision in the right external jugular vein into the superior vena cava to the level of the right atrium. The catheter was run subcutaneously to the dorsal surface of the neck, fixed there by a halved cylinder of a narrow 1 ml polyaethylene injection syringe sewn on to the skin, filled with 5% glucose solution and kept closed for 24 h before the start of the infusion.

During infusion, the rats were kept in open cylindrical plexiglass metabolic cages.
Blood pressure measurement

Following a 15 min exposure to a heat lamp, which was sufficient to raise the cage temperature to 38°C, the systolic blood pressure was measured in unanaesthetized rats by a non-invasive tail-cuff method with ultrasonic auscultation (Arteriosonde 1011, Roche) according to Reichle (1971) and Buñag (1973). During the blood pressure measurement, the infusion was continued, and the rats were kept in a heated restrainer (Narco Bio-Systems).

Blood collection and tissue incubation

At the end of the infusion period, usually between 9 and 11 a.m., 5 ml blood was withdrawn from the catheter within 3 to 6 min, and the rat was then decapitated. The adrenals of each animal were excised and kept separate in a small vessel containing ice cold incubation medium. This was a modified Krebs-Ringer bicarbonate buffer containing 3.6 mmol K⁺ and 20 g glucose per litre. Adjoining fatty tissue was removed, and the single adrenals were quartered. The tissue from each animal was put into a rubber stoppered incubation flask (25 ml) containing 6 ml of incubation medium [1,2,6,7-³H]corticosterone (Radiochemical Centre, Amersham, England; 2 µCi and 300 nmol per flask) was added in 0.06 ml ethanol (96%). Within 40 min after killing the animal, the adrenal tissue was incubated for 120 min at 37°C in an atmosphere of 95% O₂ and 5% CO₂ in a metabolic shaker. At the end of the incubation, the medium was separated from the tissue and kept frozen at −20°C. The adrenal sections were gently blotted on filter paper and weighed.

Analytical methods

Serum aldosterone concentrations were measured by radioimmunoassay after chromatographic purification according to Underwood & Williams (1972). The aldosterone antiserum utilized was a gift of the NIAMDD, National Institutes of Health, Bethesda, Md., USA.

Serum corticosterone concentrations were determined by a radioimmunoassay without chromatography utilizing a sheep antiserum (gift of Dr. Bryan Hudson, Parkville, Australia) – raised against cortisol-21-hemisuccinate coupled to porcine thyroglobulin – and [1,2,6,7-³H]corticosterone as the radioligand. The assay procedure was essentially the one used by Wang et al. (1974) for measuring plasma testosterone. At 50% displacement, the relative cross-reactivities of some possible interfering steroids were (corticosterone 100%): cortisol 20%, 11-dehydrocorticosterone 1%, 18-hydroxy-11-deoxycorticosterone 0.3%, 18-hydroxycorticosterone 10%, aldosterone 0.6%, deoxycorticosterone 5%, progesterone 4%. According to repeated assays of a pooled rat serum with a mean corticosterone concentration of 18.9 µg/100 ml, the within-assay coefficient of variation was 8.7% (N=9) and the between-assay coefficient of variation 13.1% (N=17).

Tritiated aldosterone in the incubation medium was measured by a previously described and evaluated double isotope dilution procedure (Baumann & Müller 1972a).

Serum and urinary sodium and potassium concentrations were determined by flame spectrophotometry using an internal lithium standard.
Effect of an infusion of KCl (0.154 M, 1.58 ml/h) into potassium-deficient rats upon serum concentrations of potassium, aldosterone and corticosterone, the adrenal weight (expressed in mg adrenal weight per 100 g body weight) and the conversion of tritiated corticosterone ([3H]B, 300 nmol/flask) to aldosterone (ALDO) by quartered adrenals incubated for 2 h. Mean values ± SEM are shown. Asterisk refer to the significance of the difference between adjacent points according to t-tests: * P < 0.05, ** P < 0.01, ***P < 0.001.

RESULTS

KCl infusion into potassium-deficient rats

Rats which had been kept on a potassium-deficient diet for two weeks were given intravenous infusions of 0.154 M KCl at at rate of 1.58 ml/h. As shown in Fig. 1, this led within 2 h to a significant increase in the mean serum potassium concentration from 2.9 to 4.7 mmol/l. With continued infusion, the serum potassium rose to 8.4 mmol/l within 12 h and returned to 5.3 mmol/l within 48 h. The serum aldosterone concentration rose from 6 to 28 ng/100 ml
during the first 2 h and then again from 37 to 142 ng/100 ml between 6 and 12 h of KCl infusion. This high aldosterone level remained unchanged between 12 and 48 h of infusion. The conversion of tritiated corticosterone to aldosterone increased significantly ($P < 0.001$) within 6 h and reached a value characteristic of normally fed rats (see Figs. 2 and 3) within 12 h. During the following 36 h of continued KCl infusion there was a significant additional increase in the conversion rate. Observed alterations in the serum corticosterone

![Graph showing effects of ACTH infusion on serum aldosterone, corticosterone, conversion of tritiated corticosterone to aldosterone, and relative adrenal weight.](https://www.bioscientifica.com)

**Fig. 2.**
Effect of an infusion of ACTH (50 μg/day, solid lines and dots) in 5% glucose into rats upon the serum concentrations of aldosterone and corticosterone, the relative adrenal weight and the conversion of tritiated corticosterone to aldosterone by adrenal tissue. Control animals received an iv infusion of 5% glucose (C, broken lines, open dots). Mean values ± SEM are shown. Asterisk refer to the significance of differences between experimental values and corresponding control values according to t-tests: $*P < 0.05, **P < 0.01, ***P < 0.001$. 

684
level were statistically not significant, except for a decrease between 12 and 48 h of infusion. The relative adrenal weight significantly increased (+30%) within the first 2 h and decreased again between 12 and 48 h of infusion. In control experiments not shown in Fig. 1, infusion of glucose solution for 48 h into potassium-deficient rats did not result in any changes in serum aldosterone concentration or in the conversion of corticosterone to aldosterone.

**ACTH infusion**

Synthetic β¹⁻²¹ ACTH (Synacthen®, Ciba-Geigy) was infused at a rate of 50 μg/day in 5% glucose (53 ml/day) into rats feeding on a complete diet. Control animals were given 5% glucose without the peptide. Within the first day of ACTH infusion, the mean serum aldosterone concentration rose from 12 to 100 ng/100 ml (Fig. 2). However, after 48 and 96 h of continued infusion, the serum aldosterone concentrations were no longer significantly different from the respective control values. The conversion of tritiated corticosterone to aldosterone decreased to 33%, 15% and 3%, respectively, of the base-line values after 1, 2 and 4 days of ACTH infusion. The serum corticosterone level rose from 10 to 62 μg/100 ml after 1 day and to 87 μg/100 ml after 2 days of ACTH treatment. After 4 days of continued infusion it decreased to 46 μg/100 ml; this latter value was however still significantly above the respective control value. The relative adrenal weight increased continuously during the whole

### Table 1.

Effect of treatment with ACTH on serum sodium and potassium concentrations. Mean values ± SEM. None of the differences between control animals and ACTH-treated rats was statistically significant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum concentration (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>1 day</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
</tr>
<tr>
<td>ACTH</td>
<td>7</td>
</tr>
<tr>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>ACTH</td>
<td>6</td>
</tr>
<tr>
<td>4 days</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
</tr>
<tr>
<td>ACTH</td>
<td>6</td>
</tr>
</tbody>
</table>
period of ACTH administration. The serum sodium and potassium concentrations in ACTH-treated rats were not significantly different from the values found in control animals (Table 1).

Angiotensin II infusion

In a third series of experiments, rats which had been kept on a complete diet for two weeks were put on a sodium- and potassium-deficient diet and were given an intravenous infusion of a medium containing NaCl (47.2

![Graph](image)

**Fig. 3.** Effects of an infusion of angiotensin II (20 ng/min, A II, solid lines and dots) upon systolic blood pressure, the serum concentrations of aldosterone and corticosterone, the relative adrenal weight and the conversion of tritiated corticosterone to aldosterone by incubated adrenal tissue. Control animals received the same infusion medium without the peptide (C, broken lines, open dots). Mean values ± sem are shown. Asterisks refer to the significance of differences between experimental and control values according to t-tests: *P < 0.05, **P < 0.01, ***P < 0.001).
mmol/1), KCl (45.3 mmol/1) and glucose (110.7 mmol/1 at a rate of 53 ml/day. When angiotensin II (val⁵-angiotensin II-asp¹β-amide, Hypertensin®, Ciba) was added and infused at a rate of 20 ng/min, a persistent elevation in blood pressure resulted (Fig. 3). The mean serum aldosterone concentration increased to 117 ng/100 ml after 1 day of angiotensin II administration, but returned to the control level within the second day of continued infusion. The conversion of corticosterone to aldosterone was significantly elevated by 64 % and 46 %, respectively, above control values after 1 and 2 days of angiotensin infusion. After 4 days of infusion, the difference in the conversion rate between angiotensin-treated and control animals was no longer significant. The serum corticosterone remained unchanged in control as well as in angiotensin-treated rats. In both groups of animals, the relative adrenal weight increased by 38 % within the first day of infusion and then remained constant for the rest of the experiment. Neither the urinary excretion nor the serum concentration of sodium were significantly affected by angiotensin administration (Table 2). The serum potassium concentration was moderately decreased after 1 day of angiotensin treatment. Rats receiving angiotensin for 2 days excreted significantly more potassium in the urine than control animals.

Table 2.
Effect of treatment with angiotensin II (Ang. II) on urinary excretion of sodium and potassium and on serum sodium and potassium concentrations. Mean values ± sem. In brackets P values of significance calculated by t-tests and referring to differences from control values. Other differences were not significant. Each animal received 2.5 mmol NaCl and 2.4 mmol KCl per day by iv infusion.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urinary excretion (mmol)</th>
<th>Serum concentration (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium</td>
<td>Potassium</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>2.05 ± 0.16</td>
</tr>
<tr>
<td>Ang. II</td>
<td>8</td>
<td>2.42 ± 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.91 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.24 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>3.80 ± 0.07</td>
</tr>
<tr>
<td>Ang. II</td>
<td>12</td>
<td>3.77 ± 0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.93 ± 0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.41 ± 0.16</td>
</tr>
<tr>
<td>4 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>6.16 ± 0.36</td>
</tr>
<tr>
<td>Ang. II</td>
<td>14</td>
<td>6.19 ± 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.00 ± 0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.69 ± 0.37</td>
</tr>
</tbody>
</table>

687
DISCUSSION

Studies on the biosynthesis and secretion of aldosterone in rats, involving solitary caging, anaesthesia, surgery, prolonged infusions, measurement of blood pressure and finally blood collection may be seriously hampered by unavoidable experimental stress leading to acute or chronic increases in ACTH or renin secretion. In the present experiments, acute excesses in ACTH secretion at the time of blood collection could be excluded by normal serum corticosterone levels. On the other hand, it is known that treatment with ACTH has a long-term suppressive effect on aldosterone biosynthesis (Vecsei et al. 1966b; Müller 1978). Chronic increases in endogenous ACTH secretion may have similar consequences. Although most of our cannulated control animals appeared to be quiet, relatively unrestrained and not much bothered by the infusion tubes, their adrenal weight increased by 30–40% during the first day of infusion. This, however, was not accompanied by a decrease in the adrenals' capacity to convert corticosterone to aldosterone.

In the present studies, the conversion of corticosterone to aldosterone was for practical considerations assessed in quartered adrenal glands, containing all three zones of the cortex, rather than in separate zona glomerulosa tissue (Baumann & Müller 1972a) or in adrenal mitochondria (Marusic & Mulrow 1967), which would have excluded an interference by the relatively high and variable amounts of endogenous corticosterone produced by the zona fasciculata. However, we added labelled corticosterone in a high substrate amount, which was far in excess of the expected endogenous corticosterone production of the adrenal gland1. Thus, the dilution of the added precursor by endogenous steroid should have been negligible. Sodium restriction, potassium restriction and potassium repletion, respectively, led to alterations in the conversion of tritiated corticosterone to aldosterone which were similar in extent to the alterations previously induced by these dietary measures in the capsular adrenal or mitochondrial conversion of corticosterone to both 18-hydroxycorticosterone and aldosterone. Therefore, the conversion of added corticosterone to aldosterone by complete adrenal tissue appears to be a valid parameter of the zona glomerulosa 18-hydroxylase activity.

When potassium-deficient rats were repleted by an intravenous infusion of potassium chloride, the resulting increases in the conversion of corticosterone to aldosterone occurred somewhat faster than in previous experiments in which potassium chloride had been added to the drinking fluid (Baumann & Müller 1972a). However, it still took as long as 6 h before a significant increase in the

1) We added 300 nmol of tritiated corticosterone to each pair of adrenals. According to a previous study (Müller 1978), the endogenous production of corticosterone by incubated decapsulated glands varied between 0.8 nmol (normal rats) and 1.5 nmol (ACTH-treated rats) per rat.
conversion rate could be detected and 12 h until it became normal. Thus, irrespectively of its route of administration, potassium stimulates the zona glomerulosa 18-hydroxylase activity rather slowly. This may have been reflected by the observed alterations in the serum aldosterone concentration, which moderately increased within the first 2 h and then remained stable for the next 4 h in spite of a continuous rise in serum potassium. The marked but delayed increase in serum aldosterone occurring between 6 and 12 h may indicate that a full response of the zona glomerulosa to elevated ambient potassium concentrations requires a normal or near-normal activity of the enzymes involved in the final stages of aldosterone biosynthesis.

In rats as well as in man (Muller et al. 1956; Tucci et al. 1967; Newton & Laragh 1968; Biglieri et al. 1969), sheep (Scoggins et al. 1974) and dogs (Ganong 1972) the aldosterone-stimulating effect of exogenous ACTH was transient and was no longer apparent after 2 days of constant infusion in spite of a persistent stimulatory effect of the peptide on the serum corticosterone concentration and on the adrenal weight. As mentioned above, treatment of rats with ACTH in vivo results in a suppressed aldosterone biosynthesis in vitro. The possible physiological mechanisms responsible for this phenomenon have recently been extensively discussed by one of us (Müller 1978). The results of the present study are consistent with the hypothesis that the self-limiting action of ACTH on aldosterone secretion could be due to its suppressive effects on late steps of aldosterone biosynthesis. After one day of ACTH infusion, the adrenal conversion of corticosterone to aldosterone was already markedly diminished, but must still have been sufficient to allow a high aldosterone output under maximum stimulation of early biosynthetic steps. In the course of the second day of ACTH administration the remaining zona glomerulosa 18-hydroxylase activity apparently fell below a critical level and became rate-limiting for the further output of the steroid.

On the 4th day of ACTH administration, the serum corticosterone was lower than on the second day. We have at the present no explanation for this apparent decrease in the zona fasciculata response. The continued increase in adrenal weight excludes a loss of the peptide by destruction or adsorption to the infusion tubes.

Although in earlier studies angiotensin II was found to have no aldosterone-stimulating effect in intact rats or to act only when given in excessive doses (Eilers & Peterson 1964; Cade & Perenich 1965; Marieb & Mulrow 1965; Dufau & Kliman 1968), Campbell et al. (1974) and Coleman et al. (1974) have more recently reported that small doses of angiotensin II (1–5 ng/min) consistently raised plasma aldosterone concentrations within 20 min in conscious intact sodium-replete rats. Bojesen (1966) had previously succeeded in stimulating aldosterone secretion by a 2-h infusion of angiotensin II (10 ng/min). After 24 h of continued infusion, he found plasma aldosterone still elevated but to
a lesser extent than after 2 h. In the present study, the mean serum aldosterone level was significantly elevated after 24 h of angiotensin II (20 ng/min) infusion, but the extent of stimulation varied considerably between individual rats. After 48 h of continued infusion, the serum aldosterone returned to normal whereas the blood pressure remained elevated. A similar transient aldosterone-stimulating effect of exogenous angiotensin has been previously observed in sheep by Blair-West et al. (1962). By contrast, prolonged infusions of angiotensin elicited sustained increases in aldosterone secretion in man over periods of several days (Ames et al. 1965; Oelkers et al. 1975, 1978). In dogs, the duration of the aldosterone-stimulating effect of continuously infused angiotensin II appears to be dose-dependent, with a low dose (5 ng/kg/min) inducing a transient and a high dose a persistent elevation in the plasma aldosterone concentration (McCaa et al. 1975; McCaa 1978). Since the cessation of the steroidogenic response was not associated with a decrease in the adrenal conversion of corticosterone to aldosterone, the present study yielded no explanation for the transiency of aldosterone stimulation by angiotensin II in the rat. We can only speculate that the prolonged angiotensin administration had led to a decrease in the affinity of the zona glomerulosa receptor for the peptide.

The significant – although moderate and also transient – increase in the adrenal conversion of corticosterone to aldosterone induced by angiotensin II in rats, confirms the observation made by Aguilera et al. (1975) in dogs that angiotensin in contrast to ACTH has a stimulatory action on the final steps of aldosterone biosynthesis. Boyd et al. (1971) have presented evidence indicating that the increased activity of the final steps of aldosterone biosynthesis in sodium deficiency may be due to an increase in serum potassium concentration. It is therefore possible that both an elevated plasma angiotensin II and an elevated plasma potassium contribute to the increased rate of conversion of corticosterone to aldosterone observed in sodium deficiency.

ACKNOWLEDGMENTS

This work was supported by Research Grants No. 3.018-0.73 and 3.704-0.76 of the Swiss National Foundation for Scientific Research. J.K. carried out parts of these studies as a trainee of the 8th Postgraduate Course in Experimental Medicine, University of Zurich.

We wish to thank Miss Lilian Frei, Miss Brigit Hauri, Miss Eva-Grethe Lund and Miss Idda Rutz for their excellent technical assistance. The gifts of steroid antisera by Dr. Bryan Hudson and by the NIAMDD are gratefully acknowledged. Thanks are also due to Prof. J. Vlach and Mr. P. Horn for valuable technical advice.
REFERENCES


Received on October 30th, 1978.