Effect of spironolactone on aldosterone regulation in man

R. C. GAILLARD*, A. M. RIONDEL, P. CHABERT AND M. B. VALLOTTON
Division of Endocrinology, Department of Medicine, Cantonal Hospital, University of Geneva, Switzerland

(Received 6 September 1978; accepted 16 October 1979)

Summary

1. The action of spironolactone, a well-known antagonist of mineralocorticoids, on aldosterone regulation was investigated in normal young men to see whether it also acted as an inhibitor of biosynthesis in the adrenal gland.

2. The action of spironolactone was studied under three different conditions: (a) during 3 days of treatment with spironolactone; (b) during 1 day of combined administration with long-acting adrenocorticotropic hormone (ACTH); (c) in the course of a continuous infusion of angiotensin II.

3. Spironolactone did not alter the metabolism of aldosterone or cortisol.

4. Spironolactone administration produced: (a) a marked increase in both aldosterone secretion and plasma renin activity, but no change in the plasma aldosterone/plasma renin activity ratio, the cortisol secretion rate or the plasma corticosterone concentration; (b) no blunting in the response of aldosterone to stimulation by ACTH; (c) no decrease in plasma aldosterone concentration when changes of the endogenous renin activity were prevented by an infusion of angiotensin II.

5. These results do not confirm the considerable inhibition of aldosterone excretion found by others after spironolactone administration to normal men. We observed no inhibition of aldosterone biosynthesis by spironolactone. However, a minimal, short-lived inhibition of biosynthesis cannot be excluded, but this possible action of spironolactone plays at best a minor role in the action of this drug.

Key words: adrenocorticotropic hormone, aldosterone, angiotensin II, cortisol, corticosterone, plasma renin activity, spironolactone.

Abbreviations: ACTH, adrenocorticotropic hormone; ANG II, angiotensin II.

Introduction

It has been generally recognized that spironolactone acts as a competitive antagonist of mineralocorticoid action at the kidney. The subsequent loss of sodium activates the renin–angiotensin–aldosterone system (Kagawa, 1960; Davidson, Coppage, Island & Liddle, 1961; Spark, Dale, Kahn & Melby, 1969; Fisher, Woo & Horton, 1972).

More recently inhibition of aldosterone biosynthesis after spironolactone administration was demonstrated in vitro, in adrenal preparations from various species (Erbler, 1972, 1973; Cheng, Suzuki, Sadee & Harding, 1976), and in vivo, in the early phase of treatment of primary aldosteronism (Conn & Hinerman, 1977; Vetter, Appenheimer, Lucas, Weiand, Herschbach, Glänzer, Witaszek & Krück, 1977).

Studies on the effect of spironolactone in normal men are quite limited and contradictory (Davidson et al., 1961; Erbler, 1974a,b; Abshagen, Spörl, Schöneshofer, L’Age, Rennekamp & Oelkers, 1976).

To further document the action of spironolactone on aldosterone regulation in healthy young
volunteer subjects, we studied the effect of acute administration of spironolactone on the plasma aldosterone/plasma renin relationship and on the metabolism of aldosterone. It was reasoned that if aldosterone biosynthesis was inhibited by the drug, the ratio of plasma aldosterone to plasma renin activity would decrease. However, this decrease could also result from an increase in the rate of aldosterone metabolism, since spironolactone is known to induce hepatic enzymes.

In addition, any effect of spironolactone on adrenal enzymes may be accompanied by changes in the secretion of other steroids and by a blunting of the response to stimulation. Therefore its effect on cortisol and corticosterone, as well as on the response of aldosterone to ACTH and angiotensin II (ANG II), was studied. The use of a constant stimulation of aldosterone by ANG II was judged particularly important since, in these circumstances, any counter-regulation by the endogenous system in response to salt depletion is minimized.

Material and methods

Subjects

Normal young male medical students, aged 23–28 years, were studied. They were placed on a diet with 130 mmol of Na/day the day before the study and maintained on this regimen throughout the investigations. They gave informed consent to the study, which had been approved by the Ethical Committee of the Department of Medicine.

Experimental design

Protocol 1. After a control day, 400 mg of spironolactone (Aldactone, Searle) was administered orally for 3 days to eight subjects (200 mg at 08.00 hours, 100 mg at 12.00 and 19.00 hours).

Blood samples were drawn between 07.30 and 08.00 hours for determinations of electrolytes, total proteins, aldosterone, glucocorticoids and renin activity. The subjects had been fasting and recumbent for 12 h. Twenty-four hour urine collections were made daily for 5 days for measurement of electrolytes, aldosterone and 17-ketogenic steroids.

Aldosterone and cortisol secretion rates were measured before and on the third day of spironolactone treatment in five of the subjects. Tetrahydroaldosterone was measured in two of the subjects.

Protocol 2. After a control day, 1 mg of long-acting ACTH (Synacthen Depot, Ciba) and 400 mg of spironolactone were administered for 1 day to six subjects. ACTH was given intramuscularly with the 08.00 hours dose of spironolactone, which was administered as in protocol 1; five subjects, serving as controls, received ACTH alone. Urine was collected for 2 days for the measurement of aldosterone metabolites and 17-ketogenic steroids. Tetrahydroaldosterone was measured in two of the six subjects receiving ACTH and spironolactone and in three of the controls.

Protocol 3. ANG II (Hypertensin, Ciba) in 5% glucose solution was infused at 08.00 hours in four subjects at a rate of 7 ng min−1 kg−1 for 330 min in two subjects, and for 450 min in two other subjects. Blood samples were drawn for aldosterone, glucocorticoids and renin activity determinations before infusion and then every 30 or 60 min until the end of ANG II infusion. Spironolactone (400 mg) was given orally immediately after the 90 min blood sampling.

Determinations

Aldosterone, corticosterone and renin activity were measured in plasma by radioimmunoassay, and cortisol was measured by a competitive-binding technique (Leclercq, Copinschi & Franckson, 1969; Vallotton, 1971; Underwood & Williams, 1972; Gaillard, Merkelbach, Riondel, Vallotton & Muller, 1976).

Secretion rates of aldosterone and cortisol, and urinary tetrahydroaldosterone were measured by double-isotope dilution techniques (New, Seaman & Peterson, 1969). The urinary radioactivity excreted after the injection of the labelled steroid was measured directly by liquid-scintillation counting of 1-5 ml of urine. An internal standard was used for quenching correction.

Urinary aldosterone, as 3-oxo conjugate, was measured by double-isotope dilution technique (Kliman & Peterson, 1960), or by radioimmunoassay (Langan, Jackson, Adlin & Channick, 1974). The latter was modified as indicated in the section below (Methodology controls). 17-Ketogenic steroids were measured by the procedure of Metcalf (1963). Electrolytes in plasma and in urine were determined by standard flame-photometry methods. The Wilcoxon test or, when mentioned, the Student's t-test, was applied to assess the statistical significance of the results, which are expressed as mean ± SEM.
Methodology controls

Spironolactone is known to interfere with the radioimmunoassay of some steroids, producing spuriously elevated results (Sadee, Michaels, Schmiedek & Baethmann, 1975; Tan & Mulrow, 1975).

Plasma determinations were controlled in several ways. The sequential changes in plasma aldosterone, cortisol and corticosterone concentrations were studied after a single oral dose of spironolactone (400 mg) in two healthy subjects. Karim, Zagarella, Hribar & Dooley (1976) have shown that the maximum serum concentration of ethyl acetate-extractable metabolites of tritiated spironolactone was reached 2.6 ± 0.22 h after the oral administration of 200 mg of [3H]spironolactone to healthy fasting men. During the 4 h after the administration of the drug, no spurious elevation of the normally low values was observed for aldosterone, cortisol or corticosterone in our experiment (see Clinical Science Table 79/8 deposited with the Librarian, the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, from whom copies may be obtained on request).

The aldosterone method was checked in two other ways: (a) the steroid was measured in the plasma of an adrenalectomized patient previous to and on the second day after spironolactone medication (400 mg/day); the values found were 0.03 and 0.05 nmol/l respectively; (b) the plasma of four normal young male subjects obtained after 3 days of spironolactone treatment (400 mg/day) was processed both by the routine radioimmunoassay and by the same assay with an extra purification step (paper chromatography: E₂B). No significant difference in the aldosterone concentrations, measured by both procedures, was observed.

The determination of urinary aldosterone with the radioimmunoassay with the sheep antiserum no. 088 from NIAMD, Bethesda, Maryland, U.S.A. on pH 1-hydrolysed extracts, was checked by comparison with the double-isotope dilution method. The following correlation was obtained when the urine samples of normal subjects receiving 400 mg of spironolactone/day were measured by the double-isotope dilution (x) and by the radioimmunoassay (y) methods: y = 25.1 ± 1.3x (n = 29; r = 0.923). The overestimate of aldosterone with radioimmunoassay was corrected by a paper-chromatography step (Bush B5) (y = 1.2 + 1.1x; n = 24; r = 0.952).

The specificity of the double-isotope dilution techniques was insured by the constancy of the isotope ratios obtained in the respective procedures. The validity of the aldosterone methods was also proved indirectly by the observation in four subjects that after a 4-day stimulation with long-acting ACTH (1 mg/day) combined with spironolactone (400 mg/day) both plasma and urinary aldosterone values were lower on the fourth day than on the control day (not shown).

Results

Protocol I

Three days of spironolactone administration produced: a mean weight loss of 2.75 ± 0.24 kg; a decrease in plasma sodium from 140 ± 0.6 to 135 ± 0.4 mmol/l and an increase in plasma proteins from 71.7 ± 1.3 to 79.5 ± 1.3 g/l. Plasma potassium (K⁺) increased before spironolactone administration to 4.01 ± 0.14, 4.03 ± 0.16 and 4.22 ± 0.07 mmol/l respectively after days 1, 2 and 3 of treatment. This increase was statistically significant on the last day only (P < 0.01).

The mean aldosterone secretion rate increased from 348.5 ± 53.5 to 1365 ± 235 nmol/day, but the mean cortisol secretion rate was unchanged, being 59.0 ± 3.6 and 59.9 ± 3.9 µmol/day (n = 5). The amount of radioactivity excreted in the urine was not altered by treatment: 73.4 ± 3.4 and 77.3 ± 2.2% of the [3H]aldosterone and 84.6 ± 5.1 and 89.5 ± 2.8% of the [14C]cortisol was excreted on day 1 after injection of the labelled steroid. The percentage of aldosterone excreted as 3-oxo conjugate was 8.1 ± 2.9% on the control day and 9.9 ± 1.3% on day 3 of spironolactone; the results are not statistically different.

Table 1 shows the effect of spironolactone on sodium excretion, which first increased significantly and after cessation of treatment decreased markedly. Urinary 17-ketogenic steroid and plasma cortisol concentrations remained constant. Plasma corticosterone concentration did not vary when the Wilcoxon test was used to compare separately the results of each day under medication with the mean value of day 1 and 2. With the same test and by comparing all the values under medication with the mean value of day 1 and day 2 a small but significant increase was found (0.025 < P < 0.05).

Fig. 1 shows the effect of spironolactone on plasma aldosterone and renin activity, both of
TABLE 1. Effect of 3 days of spironolactone administration in normal subjects

Mean values ± SEM for urinary electrolytes, 17-ketogenic steroids and plasma corticosteroids before and during 3 days of spironolactone administration are given. n = 8; Wilcoxon test: *P < 0.05; **P < 0.01.

<table>
<thead>
<tr>
<th>Day no...</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spironolactone (400 mg/day)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/day)</td>
<td>145 ± 6-7</td>
<td>244 ± 19.5**</td>
<td>221 ± 19.8**</td>
<td>179 ± 15.3*</td>
<td>75.9 ± 10.8**</td>
</tr>
<tr>
<td>Potassium (mmol/day)</td>
<td>88.2 ± 7.7</td>
<td>80.3 ± 5.6</td>
<td>94.6 ± 4.7</td>
<td>102.9 ± 3.9</td>
<td>124.6 ± 5.4**</td>
</tr>
<tr>
<td>17-Ketogenic steroids (µmol/day)</td>
<td>25.8 ± 3.8</td>
<td>30.6 ± 5.9</td>
<td>30.3 ± 4.8</td>
<td>34.8 ± 5.5</td>
<td>29.9 ± 2.8</td>
</tr>
<tr>
<td>Cortisol (µmol/l)</td>
<td>0.44 ± 0.05</td>
<td>0.37 ± 0.02</td>
<td>0.44 ± 0.03</td>
<td>0.43 ± 0.04</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>Corticosterone (nmol/l)</td>
<td>36.6 ± 10.4</td>
<td>23.4 ± 4.9</td>
<td>34.9 ± 6.3</td>
<td>46.5 ± 9.8</td>
<td>46.5 ± 8.9</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of spironolactone (400 mg/day) on plasma renin activity (●●●), plasma aldosterone (○○○) and the 3-oxo conjugate in eight subjects [mean results ± SEM; *P < 0.05 (significantly different from control day 1); **P < 0.01].

which increased after 1 day of treatment, the rise becoming significant respectively after 2 and 3 days. Urinary aldosterone rose promptly and continued to increase on day 4, when plasma renin activity and plasma K⁺ were at their highest values. In two subjects plasma aldosterone and renin activity measured 36 h after the final dose of spironolactone returned to their control values.

The plasma aldosterone/plasma renin activity ratios before and during spironolactone administration were 0.54 ± 0.19 and 0.79 ± 0.42 respectively. This change was not statistically significant as indicated by the paired Student’s t-test and the Wilcoxon test.

Protocol 2

After 1 mg of long-acting ACTH, urinary aldosterone increased from 35.8 ± 4.2 to 133.7 ± 24.4 nmol/day, and 17-ketogenic steroids from 38.3 ± 5.9 to 142.5 ± 13.8 µmol/day in the control group (n = 5). With simultaneous administration of spironolactone, urinary aldosterone increased from 43.8 ± 3.9 to 114.3 ± 9.2 nmol/day and 17-ketogenic steroids from 32.7 ± 4.5 to 127.0 ± 15.5 µmol/day (n = 6). These responses are not statistically different. No changes in plasma renin activity values were observed between the control values and those obtained after ACTH or ACTH and spironolactone.

The two metabolites of aldosterone in urine were measured in two subjects in protocol 1 and in five subjects in protocol 2. There was a good correlation between the variations of the two compounds. The tetrahydroaldosterone/3-oxo conjugate ratio in these seven subjects was 3.50 ± 0.50 (n = 7) in the urine collected without any
drug treatment and $4.61 \pm 0.65 \ (n = 6)$ in the urine samples collected under spironolactone treatment alone. This difference is not statistically significant. ACTH administration lowered this ratio to $2.10 \pm 0.79$ in the three subjects and the same ratio was observed in the two subjects where spironolactone was added to ACTH.

**Protocol 3**

During the perfusion with ANG II, endogenous plasma renin activity was inhibited to values lower than $0.39 \text{ pmol} \cdot \text{h}^{-1} \cdot \text{ml}^{-1}$ and the diastolic blood pressure increased by $5-15 \text{ mmHg}$. Plasma aldosterone increased and the value reached was maintained for 4–6 h after the administration of 400 mg of spironolactone. In the four subjects studied plasma aldosterone was respectively 0.74, 0.47 and 0.48 nmol/l at 90 min before the administration of spironolactone and 0.24 and 0.34 nmol/l 4 h afterwards in the first two subjects, and 0.50 and 0.39 nmol/l after 6 h in the second two subjects. Plasma cortisol and corticosterone concentrations fluctuated in parallel (see the deposited Table referred to in the Material and methods section).

**Discussion**

There are only few studies on the effect of spironolactone in normal men and their results are conflicting. Davidson et al. (1961) studied five normal subjects on a low sodium diet and did not observe a decrease of aldosterone during spironolactone administration. This result is strong evidence against the presence of a significant block induced by the drug. On the other hand, Erbler's (1974a) data suggested that aldosterone biosynthesis was blocked by spironolactone. He studied normal men whose aldosterone concentrations were progressively stimulated by a diuretic. In these individuals, administration of canrenone (another spiro- lactone) produced a 50% fall in plasma aldosterone concentration within 3 h. In another study in healthy subjects on a normal sodium intake, Erbler (1974a) found neither increase nor decrease in urinary aldosterone during 10 days of spironolactone treatment, but observed a strong rebound effect, which curiously lasted for 3–5 days when treatment ceased. From these data Erbler (1974a) concluded that spironolactone induced a block in aldosterone biosynthesis, the presence of which was also suggested by a study by Abshagen et al. (1976). Unfortunately Davidson et al. (1961) and Erbler (1974a,b) did not measure plasma renin activity and neither Abshagen et al. (1976) nor Erbler (1974a,b) measured aldosterone secretion rate or aldosterone metabolites. Without these data, it is difficult to resolve the apparently conflicting results.

In this report we present data concerning the effect of spironolactone administration in normal subjects on the relation between aldosterone and plasma renin activity, the metabolism of aldosterone and cortisol and the plasma concentrations of cortisol and corticosterone. The response of these variables to two stimuli (ACTH and ANG II) was also examined when spironolactone was given concomitantly.

We excluded any methodological interference of spironolactone or its metabolites with the assay systems used. A direct effect of a drug on the biosynthesis of a steroid in experiments in vivo can be proved only if the changes in the parameters measured to establish this effect are really due to changes in the secretion and not to changes in the metabolism of the steroid. Therefore the effect of spironolactone on aldosterone metabolism was studied with the drug being administered alone or together with ACTH. Despite the fact that spironolactone is known to induce hepatic enzymes, aldosterone metabolism was not altered. This was shown by the similar excretion pattern of the 3-oxo conjugate and tetrahydroaldosterone, by the equal rate of excretion of urinary radioactivity after injection of $^3$H-aldosterone, and by the unchanged percentage of aldosterone excreted as the 3-oxo conjugate. The constant concentrations of plasma cortisol and urinary 17-ketogenic steroids observed during medication, without changes in the cortisol secretion rate, demonstrate that cortisol metabolism was unaltered.

Three days of spironolactone administration progressively stimulated the renin–angiotensin– aldosterone system, volume depletion (as shown by the weight loss) being the most likely stimulus. Urinary aldosterone increased continuously up to the day after cessation of treatment, when plasma renin activity was still higher than on the previous day and when plasma K+ concentration was significantly increased. Therefore plasma renin activity and plasma K+ were responsible for the further urinary aldosterone increase. Thirty-six hours after the last dose of spironolactone, plasma aldosterone and renin activity had returned towards control values. During treatment plasma and urinary aldosterone showed a parallel increase (Fig. 1). The plasma
alderosterone/plasma renin activity ratio did not change significantly when compared with that on the control day. As plasma K+ rose significantly only on the last day it could not have influenced the aldosterone/renin activity ratio and produced a dissociation in their parallel increases. The effect of spironolactone on plasma corticosterone concentration was minimal.

We could not demonstrate any blunting or inhibitory effect of spironolactone on the stimulation of aldosterone by ACTH or ANG II. The same increase in urinary aldosterone was obtained when ACTH was given alone or together with the drug. Spironolactone administration during infusion of ANG II did not result in a decrease of plasma aldosterone concentrations. The infusion of ANG II and the blood sampling were performed over a period of time within which spironolactone is known to exert its effect (Ramsay, Shelton & Tidd, 1976). In our experimental design any counter-regulation by endogenous renin, and therefore ANG II, was prevented by the infusion of ANG II, and any inhibitory effect of spironolactone on aldosterone biosynthesis should have resulted in a decrease in aldosterone concentrations.

In conclusion, it has not been possible to demonstrate in man any significant inhibition of aldosterone or glucocorticoid biosynthesis after spironolactone treatment, as recently described by others. Although a minimal, short-lived inhibition, easily overcome by other stimuli, cannot be excluded, such a modest effect would in no way play a role in the mode of action of spironolactone, which acts as a competitive inhibitor of aldosterone action.

Acknowledgments

This work was supported by the Swiss National Science Foundation grant no. 3.230-0.74 and no. 3.845-0.77 and the Bickel-Birkigt Foundation. We thank Dr Mühlemann and the Searle Drug Company, Switzerland, for support and help in generously providing Aldactone. The aldosterone antiserum no. 088 was kindly provided by the Hormone Distribution Officer, NIAMD, Bethesda, Maryland, U.S.A. We acknowledge the technical assistance of Miss H. Steinort, Mrs R. Petkova and Miss V. Nicolet.

References


CHENG, S.C., SUZUKI, K., SADDE, W. & HARDING, B.W. (1976) Effects of spironolactone, canrenone and canrenolate on aldosterone and corticosterone concentration was minimal.

3.230-0.74


RAMSAY, L.E., SHELTON, J.R. & TIDD, M.S. (1976) The pharmacodynamics of single doses of prorenoate potassium and spironolactone in fludrocortisone treated normal

Acknowledgments

This work was supported by the Swiss National Science Foundation grant no. 3.230-0.74 and no. 3.845-0.77 and the Bickel-Birkigt Foundation. We thank Dr Mühlemann and the Searle Drug Company, Switzerland, for support and help in generously providing Aldactone. The aldosterone antiserum no. 088 was kindly provided by the Hormone Distribution Officer, NIAMD, Bethesda, Maryland, U.S.A. We acknowledge the technical assistance of Miss H. Steinort, Mrs R. Petkova and Miss V. Nicolet.
Spironolactone and aldosterone regulation


