The effect of spirolactone and spironolactone on plasma testosterone in man

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Summary

Both acute and chronic administration of spirolactone and spironolactone decreases plasma testosterone in man. The mechanism of this decrease was studied in a series of experiments. These studies demonstrated that the effect of spirolactone involves an increased elimination of testosterone, whereas gonadotrophin release and testicular production and release do not seem to be affected. The increased elimination of testosterone following spirolactone is apparently due neither to induction of glucuronyltransferase, sulphatase or the reductases, nor to increased conversion of testosterone to estradiol, at least not in acute experiments. The most likely explanation seems to be that spirolactone displaces testosterone from the binding plasma proteins.

Introduction

Spirolactone and spironolactone are chemically similar to progesterone and have a slight progestational activity (Hertz and Tullner, 1958). Administration of these compounds causes side effects such as gynaecomastia, impotence and menstrual disturbances. Progesterone preparations have been shown to decrease plasma testosterone in males (Gordon et al., 1970; Sundsfjord et al., 1971). A rapid decrease of plasma testosterone following spirolactone has also been reported (Dymling et al., 1972). How exactly spirolactone brings this effect about has not been elucidated. This has prompted the studies reported here.

Methods

The spirolactone used was Soludactone (canrenoate potassium, potassium-3-(3-oxo-17β-hydroxy-4,6-androstadione-17α-γ1)-propanoate) which was administered intravenously as rapid single injections in doses of 100 or 200 mg. The spironolactone used was Aldactone (3-(3-oxo-7α-acetylthio-17β-hydroxy-androst-4-en-17α-4γ1)-propanoic acid lactone).

17-Ketosteroids were determined according to the method of Vestergaard (1951) and 17-hydroxycorticosteroids as described by James and Caie (1964).

Testosterone and androstenedione in the plasma were determined using a double isotope technique (Grandy and Petersson, 1968) except for the 'estradiol study' where plasma testosterone was determined by radioimmunoassay.

The gonadotrophins, FSH and LH, and estradiol in the plasma were determined by radioimmunoassay.

Clinical material

The studies were performed in ambulatory healthy individuals and in hospitalized patients under metabolic ward conditions. Plasma samples were drawn as stated. When not indicated the studies were performed in the morning after an overnight fast.

Results

Methodological interference between spirolactone and spironolactone was studied in vitro. The results are reported in Tables 1 and 2. There were no indications of such interference. The effects of spirolactone and spironolactone on plasma gonadotrophins, FSH and LH, are presented in Tables 3 and 4. No effect could be demonstrated. The effect of spirolactone on the conversion of androstenedione to testosterone has previously been reported (Dymling et al., 1972). One additional study is reported here. It demonstrates a rapid fall of plasma testosterone in a female, without demonstrable alterations of plasma androstenedione (Table 5).

The effects of spirolactone on plasma testosterone in two males in whom the testosterone was obtained after intramuscular injections of testosterone esters are shown in Figures 1 and 2. The same rapid fall of plasma testosterone was found as in males with intact endogenous production of testosterone.

The effects of spironolactone on 17-ketosteroids and 17-hydroxycorticosteroids have previously been reported (Dymling et al., 1972). 17-Ketosteroid excretion progressively decreased, whereas 17-hydroxycorticosteroid excretion remained unaltered.

The acute effect of spirolactone on testosterone and estradiol in the plasma is shown in Table 6. The rapid fall of plasma testosterone was not accompanied by a simultaneous increase in plasma estradiol.

Discussion

Spirolactone causes a rapid, transient fall of plasma testosterone. This can in principle be an effect of decreased production or increased elimination. The aim of the reported studies was to explore which of these mechanisms is operative.

Decreased production can be due to a direct effect of spirolactone on testosterone production and/or release, an indirect effect mediated via pituitary gonadotrophins or a decreased conversion of androstenedione to testosterone. In the studies presented there were no demonstrable effects on FSH and LH. It should, however, be kept in mind that chronic administration of spironolactone caused a decrease of plasma testosterone connected with an increase of LH in five of seven healthy males (Pentikäinen et al., 1973). As a consequence, it is possible that the clinically recognized side effects, which are the results of chronic administration, are connected with additional hormonal changes, not involved in the acute changes following spirolactone.

The conversion of androstenedione to testosterone is not affected by spirolactone (Dymling et al., 1972). The effect of spirolactone on testicular production and release has not been studied directly but such an effect seems highly unlikely. In two patients in whom plasma testosterone was obtained after intramuscular injections spirolactone caused an analogous decrease of plasma testosterone.

It is therefore concluded that the decrease of plasma testosterone following spiro-

Table 1. Plasma from a 41-year-old healthy male was analyzed before and after in vitro addition of 1 mg of spirolactone per 10 ml.

	Testosterone μg/100 ml	Androstenedione μg/100 ml
Plasma	0.72	0.16
	0.72	0.15
Plasma + spirolactone	0.69	0.15
	0.72	0.14

Table 2. Plasma samples from J.F.D., 41-year-old healthy male, and from E.J., 53-year-old male with essential hypertension on spironolactone 200 mg daily perorally, were analyzed separately and after mixing. Each sample was analyzed twice.

	Testosterone μg/100 ml	Androstenedione μg/100 ml
J.F.D.	0.67	0.09
	0.63	0.10
E.J.	0.54	0.11
	0.52	0.10
J.F.D. + E.J.	0.51	0.09
	0.62	0.10

nolactone administration is not an effect on testosterone production but rather an effect on testosterone elimination. Testosterone is metabolized by the liver enzymes glucuronyltransferase and sulphatase 5β-reductase, and converted to estradiol. 17-Ketosteroids progressively decrease during spironolactone administration (Nocke et al., 1971; Dymling et al., 1972; Pentikäinen et al., 1973), which has been interpreted as a competitive inhibition of steroid-conjugating enzymes (Nocke et al., 1971). The results do not imply an increased elimination of testosterone by the liver. Furthermore spirolactone does not influence the prostate 5α-reductase activity (Corvol et al., 1976).

The very limited number of observations reported here did not demonstrate any acute effect of spirolactone on the conversion of testosterone to estradiol. However, two weeks treatment with spironolactone caused an increase in urinary output of 17β-estradiol as well as estriol and estrone (Pentikäinen et al., 1974). Also the blood estradiol levels increased in six patients treated with spironolactone who developed gynecomastia (Rose et al., 1977). However, an increased conversion of testosterone to estradiol may become apparent only on chronic administration of spironolactone but cannot explain the acute effect on plasma testosterone.

The remaining possibility is a competitive displacement of testosterone from testosterone-binding plasma proteins. Such a displacement has been demonstrated by Caminos-Torres et al. (1977). An increased metabolic clearance rate of testosterone has also been demonstrated. These two facts could be connected since testosterone itself increases the metabolic clearance rate of testosterone (Southren et al., 1973). The sequence of events could consequently be the following: Spironolactone displaces testosterone from protein binding sites. The free testosterone causes an increased metabolic clearance rate of testosterone. This does not seem to occur via either of the established pathways of testosterone metabolism. One possibility is increased excretion in the urine. Measurements of urinary excretion of testosterone have to our knowledge not

Table 3. FSH and LH in 3 menopausal women (B.J., U.N. and B.R.), one woman with idiopathic oedema (I.G.) and 3 males, one with primary hypogonadism (C.S.), one with renovascular hypertension (D.P.) and one with Addison's disease

Time in hours	Spirolactone (mg i.v.) spironolactone (mg p.o.)	B.J. F 55 yr		U.N. F 51 yr		B.R. F 58 yr		I.G. F 36 yr		C.S. M 19 yr		D.P. M 50 yr		M.A. M 71 yr	
		FSH (ng/ml)	LH (ng/ml)	FSH (ng/ml)	LH (ng/ml)	FSH (ng/ml)	LH (ng/ml)	FSH (ng/ml)	LH (ng/ml)	FSH (ng/ml)	LH (ng/ml)	FSH (ng/ml)	LH (ng/ml)	FSH (ng/ml)	LH (ng/ml)
0	200 mg i.v.	10.4	5.9	11.5	6.1	7.8	3.5	4.0	1.6	17.5	4.5	6.2	2.6	2.4	2.1
0.5		10.8	5.0	9.7	5.3	–	–	3.9	2.3	15.9	4.2	5.1	2.1	2.7	2.1
1.0		12.0	5.1	9.0	5.4	–	–	4.4	1.7	17.6	4.6	5.4	1.9	2.5	2.1
2.0		14.2	4.5	10.0	5.7	–	–	3.6	1.7	15.8	3.7	5.8	1.4	2.9	2.7
4.0		14.5	5.3	10.2	6.8	–	–	3.9	1.7	16.9	5.3	–	–	2.5	1.8
8.0		12.1	5.3	10.1	4.8	–	–	3.8	1.4	14.6	3.8	8.1	1.3	2.7	1.9
12.0	200 mg i.v.	11.1	5.2	15.7	5.0	–	–	3.3	1.8	–	–	–	–	2.4	1.9
24.0	150 mg p.o.	11.8	4.4	14.9	5.4	10.5	3.6	4.3	1.8	13.1	4.1	6.5	1.6	2.7	1.9
48	150 mg p.o.	12.3	5.4	14.3	6.3	10.2	3.8								
72	150 mg p.o.	12.1	5.4	13.4	5.8										
96	150 mg p.o.	–	–	15.0	5.3	7.4	6.0								
120	150 mg p.o.	–	–	12.8	5.8										
144	150 mg p.o.	–	–	17.0	6.4	11.6	3.7								
168	150 mg p.o.	12.9	4.1	12.5	5.5	8.6	4.9								

Table 4. FSH and LH in a 58-year-old healthy female. Spirolactone, 200 mg intravenously, was given at 0900 hours

Time	FSH (ng/ml)	LH (ng/ml)
0830	12.5	11.8
0855	11.6	11.5
0905	12.5	11.4
0910	12.5	13.25
0915	12.5	11.4
0930	11.1	10.9
1000	9.3	12.2
1100	11.6	10.41
1300	8.3	8.7

Table 5. p-Testosterone and p-androstenedione in a 25-year-old female with idiopathic oedema. Spirolactone, 100 mg intravenously, was administered at 0800 hours

Time	P-testosterone (μg/100 ml)	P-androstenedione (μg/100 ml)
0759	0.024	0.084
0815	0.012	0.063
0830	0.008	0.084
0900	0.023	0.055
1000	0.016	0.060
1200	0.012	0.068
1600	0.001	0.052
2000	0.008	0.041

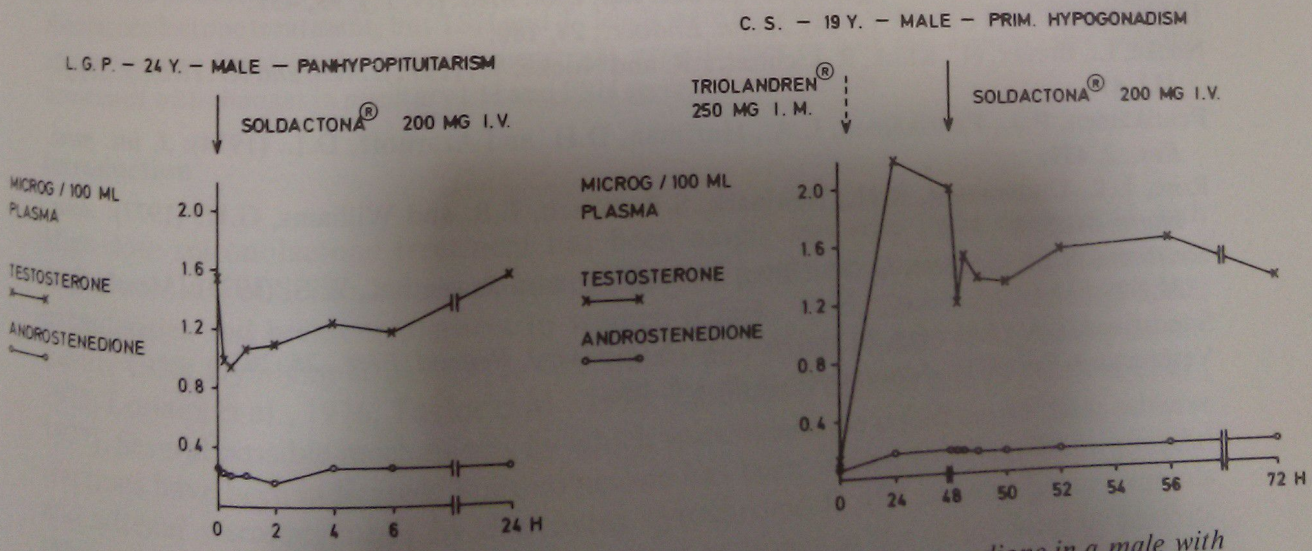

Fig. 1. The effect of spironolactone on plasma testosterone and androstenedione in a male with panhypopituitarism, where the testosterone was obtained after intramuscular injections of testosterone esters.

Fig. 2. The effect of spirolactone on plasma testosterone in a male with primary hypogonadism and exogenously administered testosterone.

Table 6. p-Testosterone and p-estradiol in a healthy male (R.A.) and a male with cardiac decompensation. Spirolactone: 200 mg intravenously, was given on day 2 at 1200 hours

	R.A. M 54 yr				F.Ö. M. 73 yr			
	Testosterone (nmol/l)		Estradiol (pmol/l)		Testosterone (nmol/l)		Estradiol (pmol/l)	
Time	day 1	day 2	day 1	day 2	day 1	day 2	day 1	day 2
0800	20	18	70	130	31	26	190	60
1159	16	15	100	60	25	12	110	110
1215	16	8.0	100	90	20	7.0	90	100
1230	14	8.0	90	50	22	6.0	140	130
1245	14	9.0	60	90	21	7.0	90	130
1300	15	8.0	70	110	22	13.0	140	140
1400	12	8.0	70	70	19	11	90	110
1600	15	11	70	60	19	11	110	110
2000	15	10	80	110	19	10	110	110
2400	15	12	90	110	20	17	120	130

been performed in acute experiments. Pentikäinen et al. (1974) found no alteration in urinary testosterone after two weeks of treatment with spironolactone.

References

Caminos-Torres, R., Ma, L. and Snyder, P.J. (1977): *J. clin. Endocr., 45,* 255.

Corvol, P., Mahoudeau, J.A., Valcke, J.-C., Ménard, J. and Bricaire, H. (1976): *Nouv. Presse méd. 5,* 691.

Dymling, J.-F., Nilsson, K.O. and Hökfelt, B. (1972): *Acta endocr. (Kbh.), 70,* 104.

Gandy, H.M. and Petersson, R.E. (1968): *J. clin Endocr., 28,* 949.

Gordon, G.G., Southren, A.L., Tochimoto, S., Olivo, J., Altman, K., Rand, J. and Lemberger, L. (1970): *J. clin. Endocr., 30,* 449.

Hertz, R. and Tullner, W.W. (1958): *Proc. Soc. exp. Biol. Med. (N.Y.), 99,* 451.

James, V.H.T. and Caie, E. (1964): *J. clin. Endocr., 24,* 180.

Nocke, L., Breuer, H., Klink, R. Lichton, I.R. and Nocke, W. (1971): *Acta endocr. (Kbh.), Suppl. 152,* 41.

Pentikäinen, P.J., Pentikäinen, L.A., Huffman, D.H. and Azarnoff, D.L. (1974): *J. int. med. Res., 2,* 439.

Rose, L.I., Underwood, R.H., Newmark, S.R., Kisch, E.S. and Williams, G.H. (1977): *Ann. intern. Med., 87,* 398.

Southren, A.L., Gordon, G.G., Olivo, J., Rafii, F. and Rosenthal, W.S. (1973): *Metabolism, 22,* 695.

Sundsfjord, J.A., Aakvaag, A. and Norman, N. (1971): *J. Reprod. Fert., 26,* 263.

Vestergaard, P. (1951): *Acta endocr. (Kbh.), 8,* 192.

Discussion

DR. FERRISS (Cork): I would like to supplement Professor Oelkers' remarks with reference to primary hyperaldosteronism. We shall later be showing data comparing the effects of spironolactone in a dose of 400 mg a day and amiloride in a dose of 40 mg a day given for six weeks. Although the rise in plasma concentrations of renin and angiotensin was more marked during spironolactone, compared with amiloride, the rise in plasma aldosterone was less marked and much less consistent. Perhaps this fits in with your data and would suggest interference with aldosterone biosynthesis directly.

DR. ROBERTSON (Glasgow): I should like to illustrate this query, which is addressed to Dr. Lewis and Professor Oelkers. Some years ago when we collaborated with Professor Oelkers we were able to show in normal human beings that when they were sodium depleted there was an enhanced aldosterone response to administered agiotensin. We have recently extended these studies, in collaboration with Dr. Nicholls, in the dog. We have been able to subject the dog to much more extreme changes in sodium balance than we could achieve in man. Figure 1 shows that on the vertical axis on a linear scale are plasma aldosterone levels, and on a log scale on the horizontal axis you will see plasma angiotensin II values. When angiotensin is infused, there is the expected rise in both plasma angiotensin II and plasma aldosterone. It is apparent that moderate sodium depletion in the dog, as in man, will enhance the aldosterone response to a given plasma level of angiotensin II. In sodium-loaded dogs we observed depression of

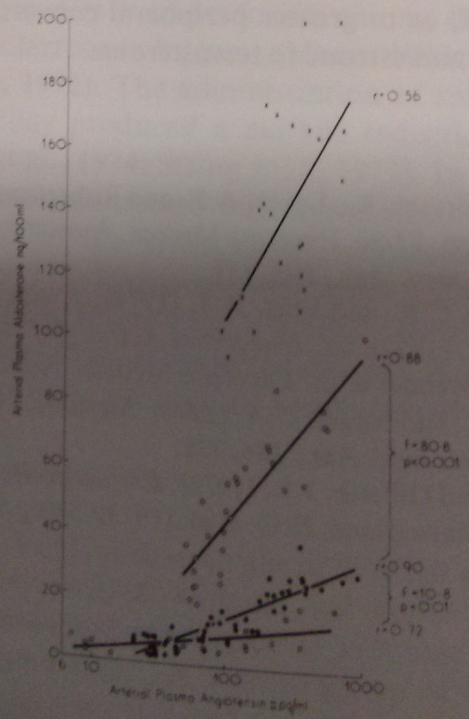

Fig. 1.

the basal levels of both angiotensin II and aldosterone, and also a much more flat dose-response curve. Up at the top is the rather more irregular but still enhanced aldosterone response of dogs which are extremely sodium depleted. My query to Dr. Lewis is whether any of the differences he saw in long-term spironolactone therapy might be due to sodium depletion and an altered relationship of this kind; and to Professor Oelkers whether he has put the data which he obtained in those normal people on aldosterone and spironolactone on to dose-response curves of this kind to see just how they fitted in with his earlier data. We have recently, in collaboration with Dr. Gordon, done similar studies in the dog where we have administered potassium and ACTH in different stages of sodium balance; and with potassium and ACTH, as with angiotensin, when you change sodium balance you can similarly change the dose response relationship in a manner very similar to that shown here.

DR. LEWIS (London): Ken et al. (*J. clin. Endocr.*, 1975, *40*, 116), when they first described the tetracosactin stimulation test that we modified, showed that sodium depletion indeed did reduce the threshold of aldosterone to ACTH and increased the maximal response. In our study, the correlation of the maximal response of aldosterone with renin levels does not reach statistical significance; it does reach significance against renal aldosterone excretion, which itself correlates significantly with renin. Also, with regard to 18-hydroxy DOC, Tuck et al. (*J. clin. Endocr.*, 1977) showed that 18-hydroxy DOC secretion was stimulated both by angiotensin II and by sodium depletion. He presumed that the sodium depletion was angiotensin II-dependent. As far as cortisol is concerned, Genest et al. (*Circulat. Res.*, 1961, *9*, 775) and Bartter and Kaplan (*J. clin. Invest.*, 1962, *41*, 715) have also shown that cortisol, corticosterone and aldosterone secretion might be stimulated by angiotension II again. Our hypothesis is that it is probably angiotensin II plus or minus low sodium and high potassium working at early stages of steroid synthesis which cause this shift in sensitivity to ACTH. However, I cannot explain why the maximum output of cortisol is not different in the two groups, whilst that of 18-hydroxy DOC is. But I agree very much with you that it does seem that sodium and the renin-angiotension system are involved.

DR. OELKERS (Berlin): I have not plotted our data on this diagram because I think the degree of sodium depletion in the normal subjects was much smaller; it was between 150 and 200 mEq altogether, and in the spironolactone group it was between 400 and 500, so I think the degree of sodium depletion is very important. But these were dogs, and we cannot compare man with dogs. Altogether I think the effect of spironolactone in clinical dosages on aldosterone synthesis is rather small. It is probably an effect which disappears after some time of treatment. If it is a true effect it would be a nice effect, especially since we have seen from Dr. Lewis' data that spironolactone does not seem to do any harm to the adrenal gland with long-term treatment since the steroids react so positively to injection of ACTH. If it was an additional effect to that on the kidney if would at least be favourable in patients with primary hyperaldosteronism, because the degree of secondary hyperaldosteronism induced by spironolactone treatment, according to sodium loss, would be blunted.

DR. CORVOL (Paris): I would like to make a comment on Dr. Dymling's presentation. We were able to confirm your results three years ago on the lowering of plasma testosterone following canrenoate administration, and we also did not find any changes in LH and FSH. Together with Dr. J. Mahoudeau, we have performed testosterone metabolic clearance rate under constant infusion of testosterone under canrenoate

administration. We found an increase in testosterone metabolic clearance rate, which would fit well with either a displacement of testosterone from TeBG or an increase in the testosterone metabolic clearance rate by a direct action of canrenoate on the liver. However, we were a bit puzzled because when we incubated in vitro TeBG with tritiated dehydrotestosterone we could not find a good competition between dehydrotestosterone and several spironolactone metabolites. I wonder if you have done any in vitro competition experiments between dihydrotestosterone or testosterone and several spironolactone compounds on TeBG?

DR. DYMLING (Malmö): No, we have not done any such experiments.

DR. AAKVAAG (Oslo): We did our competition studies with testosterone and canrenone but not with dihydrotestosterone, and as I reported previously, canrenone competes effectively with testosterone for binding to serum protein. Dihydrotestosterone has an affinity for TeBG about three times the affinity of T, and therefore it may be difficult to show this competition using dihydrotestosterone.

DR. FRASER (Glasgow): Yesterday I described some experiments in normal people treated with spironolactone in which there were rises in plasma aldosterone and parallel rises in 18-hydroxycorticosterone very similar in ratio to those we find in normal sodium-depleted subjects. We found no changes in 18-hydroxydeoxycorticosterone levels, and again this is in agreement with our studies of sodium depletion. Like Professor Oelkers, we found a rise in deoxycorticosterone concentration and a later rise in plasma corticosterone in two subjects treated with spironolactone. Plasma deoxycorticosterone levels increased to about twice normal but corticosterone remained within the normal range.

DR. S.A.S. TAIT (London): I should like to make one small comment on Professor Oelkers' work in that he mentioned our results presented in Session I. The action of angiotensin II in the rat is specifically on the zona glomerulosa and one would not expect, therefore, to see differences in deoxycorticosterone and corticosterone levels when looking at the secretion from the whole gland. However, in species where the angiotensin II does have an effect on the zona fasciculata as shown by Peytremann and by Vallotton, one sees a marked increase in cortisol. What Professor Oelkers' results suggest is that perhaps we are seeing an effect of specific stimulation of the corticosterone pathway of the zona fasciculata, and I should like to ask him have any of these studies been done with dexamethasone suppression where it could be shown whether you are in fact dealing with the zona fasciculata or the zona glomerulosa?

DR. OELKERS (Berlin): No, we have not done studies in dexamethasone-suppressed subjects. You are right in criticizing my interpretation of the DOC data because this would mean that a very large overproduction of the zona glomerulosa of DOC would have to occur in order to account for these large increases of 200-300%. When talking about steroid secretion in rats, you showed that some of the peripheral DOC is also derived from the zona glomerulosa. But if an increase by 200-300% from the zona glomerulosa occurred it could probably not account for this strong increase in plasma DOC level. This was just an attempt to interpret data but I am sure they are not fully correct.