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Effect of Spironolactone on the Renin-Aldosterone System in Rats

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PRA, PRC and the plasma concentration of aldosterone (Aldo) were measured in rats (Sp-rats) receiving a daily sc injection of Spironolactone, (Sp, 20 mg in olive oil) and in control rats (C-rats) receiving olive oil only. Animals were studied one day after starting treatment, 5 days on treatment or after 5 weeks on the study. PRA, PRC and Aldo were significantly increased in Sp-rats as compared to C-rats throughout all the study. In additional Sp-rats and C-rats, the urine volume, serum Na^+ and K^+ concentration, Na^+ and K^+ intake and the urinary excretion of Na^+, K^+ and aldosterone-18-glucoronide (UAldV) were serially measured during 5 weeks. The total radioactivity plasma clearance after an i.v. bolus injection of ^3H-aldosterone was subsequently measured in (5 Sp-rats and 5 C-rats). No significant differences in serum Na^+ and K^+ concentration and in Na^+ and K^+ balance were observed between Sp-rats and C-rats. UAldV was significantly higher in Sp-rats than in C-rats during all the study. After 5 weeks on treatment the total radioactivity plasma clearance was significantly higher in Sp-rats than in C-rats. These results indicate that Sp, at high dosage, stimulates renin release and aldosterone secretion by a mechanism unrelated to alterations in Na^+ and K^+ balance.

Keys words: Spironolactone, Aldosterone metabolism, Sodium balance.

Spironolactone (Sp), a diuretic widely used in the management of patients with edema, is a powerful antagonist of aldosterone in peripheral organs. For many years the pharmacological action of the drug was considered to be exclusively due to its ability to antagonize the effect of aldosterone on the distal nephron. However, ERBLER (9) by demonstrating that Sp markedly reduces aldosterone biosynthesis *in vitro*, suggested that inhibition of aldosterone secretion could also play a contributory role. After this study, several *in vitro* and *in vivo* investigations have tried to confirm that Sp directly influences aldosterone metabolism. *In vitro* studies have constantly found that Sp diminishes aldosterone biosynthesis (3, 7, 8, 14). However *in vivo* studies show conflicting fin-

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dings. While some investigations found that Sp causes a marked reduction of plasma aldosterone concentration (Aldo) and urinary excretion of aldosterone (5, 26, 29), other studies reported opposite results (1, 10, 12, 28). These *in vivo* studies, however, show methodological problems that limit the interpretation of the data. They were performed in conditions of normal Na^+ intake. Under this circumstance Sp may increase Na^+ excretion, contract the extracellular fluid volume and stimulate renin release. Therefore the increased plasma and urinary aldosterone observed in some of these studies could be secondary to volume depletion. On the other hand in most of these investigations aldosterone metabolism was assessed by measuring plasma concentration or urinary excretion of aldosterone, parameters that are influenced not only by changes in biosynthesis and secretion but also by changes in the metabolic clearance rate.

The present study was aimed to further investigate the effect of Sp on aldosterone levels in rats. The study was performed in conditions of high Na^+ intake to prevent the diuretic effect of Sp. Aldosterone metabolism was estimated by the total radioactivity plasma clearance after an i.v. bolus injection of ^{3}H-aldosterone. Sodium and K^+ balance, PRA and PRC were measured to assess if the effect of Sp on aldosterone metabolism could be mediated by changes in these parameters.

Materials and Methods

Animals.— The study was performed on male Wistar rats weighing 180-200 g at the beginning of the study. Animals were fed *ad libitum* with a normal chow (125 mEq of Na^+ and 178 mEq of K^+ per Kg of food) and received a saline solution (40 mEq of Na^+/l) as drinking fluid. Sp (Searle Laboratories, Madrid) was dissolved in olive oil (40 mg/ml) and the experimental rats (Sp-rats) were daily administered s.c. 0.5 ml of this solution. Control rats (C-rats) only received olive oil.

Protocol A.— Effect of Sp on PRA, PRC and plasma aldosterone concentration (Aldo). The protocol was performed in 30 Sp-rats and in 30 C-rats. Ten rats from each group were killed by decapitation one day after starting treatment and ten other rats after 5 days of treatment. Finally, the remaining rats were decapitated after 5 weeks of treatment. The time elapsed from the removal of the animal from the cage till decapitation was always less than 30 s. During exsanguination blood samples were collected under ice in K-EDTA tubes for PRA, PRC and Aldo. They were centrifuged at 4 °C and the plasma frozen at −30 °C until assayed.

Protocol B.— Effect of Sp on aldosterone metabolism. The study was performed in 10 Sp-rats and in 10 C-rats. Animals were placed in individual metabolic cages and, after one week for adaptation, the urine volume, urinary excretion of aldosterone-18-glucuronide (UAldV), Na^+ and K^+ intake and urinary Na^+ and K^+ excretion were determined sequentially following methods previously described (15). Treatment with Sp or olive oil was initiated after the week of adaptation. Measurements were perfomed on three consecutive days (Tuesday, Wednesday and Thursday) during five weeks. The fecal Na^+ and K^+ excretion were not measured. Plasma Na^+ and K^+ concentration were measured once a week (Friday) on blood samples taken under light ether anesthesia from the cavernous sinus using a micropipette.

On the sixth week after starting the protocol, the total radioactivity plasma clearance after an i.v. bolus of ^{3}H-aldosterone was determined on 5 Sp-rats and in 5 C-rats using chromatographically purified D-(1,2-^{3}H)-aldosterone (Amersham) with a specific radioactivity of 52.3 Ci/mmol. Labeled aldosterone was dissolved in 0.14 M NaCl under nitrogen just

prior to use and a dosage of 1 μCi/rat in 0.5 ml was given. Rats were anesthetized with ether and the external jugular vein and the right femoral artery were exposed and cannulated with a PE-50 catheter. ^{3}H-aldosterone was injected i.v. over a period of less than 10 s. At 0, 5, 10, 20, 30 and 60 min postinjection times, arterial blood samples (0.2 ml) were drawn. Blood was immediately centrifuged at 3000 g for 20 min at 4 °C and the total plasma radioactivity was counted in a liquid scintillation spectrometer (SL 4000 Intertechnique, Paris) using Supersolve X (Kocklight Ltd) as scintillating fluid. The total radioactivity plasma clearance was estimated from the plasma disappearance curve of the total ^{3}H-radioactivity assuming that it follows a biexponential model. Kinetic constants were calculated by using a computer program (19).

Measurements and statistical analysis.— Sodium and K^+ concentrations were measured by flame photometry (Corning EE1 450, Scientific Instruments).

The urinary aldosterone concentration was measured in urine samples (0,5 ml) adjusted to pH 1 with 1 ml of 0,2 N HCl and kept during 20 h at 30 °C. Using this procedure most aldosterone-18-glucuronide is transformed into aldosterone. Aldosterone concentration and Aldo were then measured with a highly specific RIA (CIS, Sorin Biomedica, Saluggia Italy). The coefficient variation was 10 % for intraassay and 14 % for interassay determinations. To confirm that aldosterone RIA had negligible cross reactivity with Sp or their metabolites the (UAldV) was measured in ten adrenalectomized male Wistar rats before and during a 5 day period in which animals were treated with a daily s.c. injection of 20 mg of Sp. Urinary aldosterone-18-glucoronide was undetectable in all animals throughout the study.

PRA and PRC were measured following a previously described method (16). Briefly, PRA was estimated by measuring the generated angiotensin I (AI) after 30 m incubation at pH of 7,4 and 37 °C under conditions which inhibited further conversion of AI to AII. PRC was estimated by incubating a small volume of the test plasma with a large and fixed amount of homologous renin-substrate under conditions which inhibited further conversion of AI. Pooled plasma from 36 h nephrectomized male rats were used as source of substrate. AI concentration was measured by RIA (CIS, Sorin Biomedica). The percentage of AI recovery in this assay ranged between 80 % and 97 %.

Data are presented as means±SEM. Comparison between groups was performed using the Student's *t* test or the nonparametric test of Mann-Whitney.

Results

Protocol A.— Spironolactone administration was associated with an increase in plasma levels of renin and aldosterone. One day, five days and five weeks after starting the protocol Sp-rats showed significantly higher levels of Aldo, PRA and PRC than the C-rats did (table I).

Protocol B.— Sp-rats showed significantly higher values of UAldV than the C-rats did during all the study (fig. 1). This difference was remarkably evident from the first day after starting the protocol (figure 2), which is consistent with the results of protocol A showing increased Aldo in Sp-rats as compared to C-rats within the first day of Sp administration. Figure 3 shows the total radioactivity plasma clearance after the i.v. injection of ^{3}H-aldosterone obtained in 5 Sp-rats and 5 C-rats. The total radioactivity plasma clearance was significantly increased in Sp-rats as compared to C-rats.

Table II shows urine volume, Na^+ and K^+ intake, urinary excretion of Na^+ and K^+ and the serum Na^+ and K^+ concen-

Table I. *Plasma aldosterone concentration (Aldo), plasma renin activity (PRA) and plasma renin concentration (PRC) in spironolactone treated rats (Sp-rats) and in control rats (C-rats) included in protocol A.*

	Aldo (pg/ml)	PRA (ng/ml.h)	PRC (ng/ml.h)
1 day of treatment			
Sp-rats	642 ± 88 **	3.8 ± 0.7 *	24.1 ± 6.2 *
C-rats	334 ± 35	1.3 ± 0.3	8.7 ± 2.5
5 days of treatment			
Sp-rats	833 ± 33 ***	3.4 ± 0.3 ***	15.1 ± 1.8 *
C-rats	360 ± 80	1.6 ± 0.3	8.3 ± 1.5
5 weeks of treatment			
Sp-rats	970 ± 46 ***	3.0 ± 0.6 **	14.3 ± 4.8 *
C-rats	371 ± 33	0.7 ± 0.1	5.6 ± 0.7

* $p < 0.05$; ** $p < 0.005$ and *** $p < 0.001$ with respect to control values.

tration throughout the study in rats included in protocol B. There were no significant differences between Sp-rats and C-rats for any of these parameters, the high sodium diet preventing therefore, the diuretic and natriuretic effects of spironolactone.

Discussion

The results of the current study clearly show that Sp, administered at high dosage, produces striking changes in aldosterone metabolism and plasma and urinary aldosterone levels in normal rats. Aldo and UAldV increased markedly in all animals on the first day after Sp administration and remained elevated during the five week long study. The effect of Sp on aldosterone was unrelated to interferences on the aldosterone assay, since aldosterone could not be detected in urine in adrenalectomized rats receiving a high dosage of Sp. Five weeks after starting treatment the metabolic clearance rate of al-

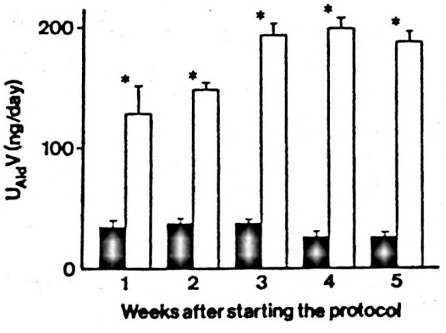

Fig. 1. *Urinary aldosterone-18-glucuronide excretion (UAldV) in control rats (shadowed bars) and in spironolactone treated rats (empty bars) included in protocol B.*
* $p < 0{,}001$, with respect to values of control rats.

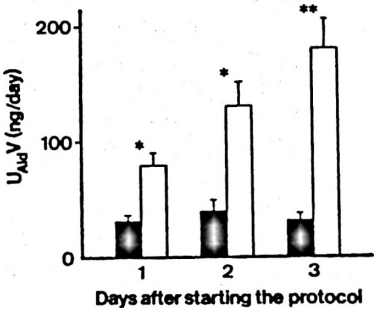

Fig. 2. *Urinary aldosterone-18-glucuronide excretion (UAldV) during the first three days after starting the protocol in control rats (shadowed bars) and in spironolactone treated rats (empty bars) included in protocol B.*
* $p < 0{,}005$, ** $p < 0{,}001$; with respect to values of control rats.

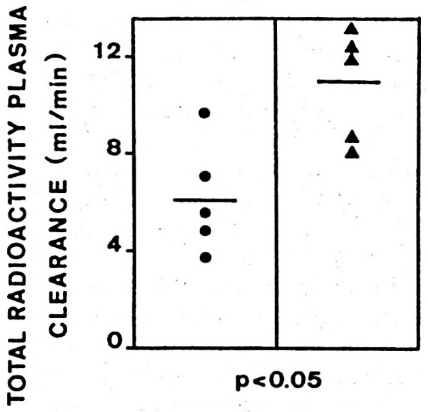

Fig. 3. *Individual values of the total radioactivity plasma clearance obtained in control rats (●) and in spironolactone treated rats (▲) included in protocol B.*

dosterone, as estimated by the total radioactivity plasma clearance after an iv bolus of ^{3}H-aldosterone was significantly increased in Sp-rats as compared to C-rats. These results suggest that the increased Aldo and UAldV in Sp-rats were not due to a defective aldosterone metabolism but to an increased aldosterone secretion.

Several mechanisms may explain the effect of Sp on aldosterone metabolism observed in the current study. Most circulating aldosterone is metabolized by the hepatic microsomal enzymes (13,21) and it is well known that this enzymatic system is markedly induced by Sp (2,4,11, 20). In addition, microsomal enzyme inducers increase the hepatic blood flow which is another important factor influ-

Table II. *Urine volume, sodium intake, urinary sodium excretion, potassium intake, urinary potassium excretion and serum sodium and potassium concentration in spironolactone treated rats (Sp-rats) and in control rats (C-rats) included in protocol B.*

	Weeks of treatment				
	1	2	3	4	5
Urine volume (ml/day)					
Sp-rats	16.4 ± 1.7	15.5 ± 0.9	14.9 ± 1.4	17.4 ± 1.6	14.1 ± 2.4
C-rats	14.7 ± 1.0	14.1 ± 1.1	13.8 ± 1.1	16.2 ± 1.5	17.7 ± 2.4
Sodium intake (mEq/day)					
Sp-rats	3.31 ± 0.22	3.37 ± 0.17	3.43 ± 0.22	3.09 ± 0.13	3.28 ± 0.15
C-rats	3.08 ± 0.13	3.23 ± 0.19	3.22 ± 0.21	3.49 ± 0.17	3.59 ± 0.14
Sodium excretion (mEq/day)					
Sp-rats	3.18 ± 0.17	3.12 ± 0.18	3.14 ± 0.13	2.78 ± 0.18	2.99 ± 0.18
C-rats	2.98 ± 0.23	2.97 ± 0.19	2.85 ± 0.19	3.09 ± 0.16	3.25 ± 0.11
Potassium intake (mEq/day)					
Sp-rats	3.14 ± 0.17	3.05 ± 0.15	3.19 ± 0.12	2.96 ± 0.23	3.72 ± 0.32
C-rats	3.00 ± 0.20	3.25 ± 0.14	3.14 ± 0.21	3.48 ± 0.14	4.08 ± 0.36
Potassium excretion (mEq/day)					
Sp-rats	2.84 ± 0.10	2.72 ± 0.08	2.95 ± 0.12	2.72 ± 0.14	3.53 ± 0.13
C-rats	2.68 ± 0.09	2.87 ± 0.11	2.90 ± 0.14	2.84 ± 0.12	3.77 ± 0.20
Potassium concentration (mEq/day)					
Sp-rats	138 ± 1.0	137 ± 0.8	137 ± 0.4	137 ± 1.3	136 ± 1.2
C-rats	137 ± 3.1	138 ± 0.5	137 ± 0.5	137 ± 0.1	136 ± 1.7
Serum potassium concentration (mEq/l)					
Sp-rats	4.4 ± 10.3	4.4 ± 0.5	4.1 ± 0.0	4.1 ± 0.2	4.2 ± 0.7
C-rats	4.2 ± 0.3	4.1 ± 0.7	4.2 ± 0.1	4.0 ± 0.2	4.1 ± 0.1

encing aldosterone metabolism (24). Sex hormones have important effects on the hepatic aldosterone metabolism (22,23). Consequently, the effect of Sp on aldosterone metabolism may also be related to the antiandrogenic properties of the drug. Finally, the hepatic metabolism of aldosterone has been shown to markedly increase in rats after 15 minutes of Sp administration (29). This rapid effect cannot be explained by any of the above mentioned processes since it takes several days for them to occur. Therefore mechanisms other than enzyme induction and endocrine dysfunction also contribute to the effect of Sp on aldosterone metabolism.

Spironolactone produced a three fold increase in PRA and PRC. This effect was apparently unrelated to alterations in Na^+ or K^+ balance since rats treated and non treated with Sp did not differ significantly with respect to Na^+ and K^+ intake, serum electrolyte concentration and urinary Na^+ and K^+ excretion. Although fecal Na^+ and K^+ excretion were not measured here, a recent study has shown that large doses of Sp do not modify these parameters in rats receiving a normal Na^+ diet (6). Therefore it is unlikely that our results could be explained on the basis of changes in fecal electrolyte excretion. Interestingly enough, the PRA and PRC increase started within the first day of Sp administration, which further supports the contention that Sp increases PRA and PRC by a mechanism independent of changes in intravascular volume. There are several investigations showing that Sp or its metabolite canrenone markedly increases the urinary excretion of prostaglandin E_2 (17, 18), which is considered to estimate the renal production of this compound. Since prostaglandin E_2 stimulates the renal release of renin, the effect of Sp on PRA and PRC may be mediated by prostaglandins. Recently RAPELLY *et al.* (25) have shown that Sp adminstration induces a sharp increase in total renin and a significant reduction in inactive renin. The effect of Sp on PRA and PRC, which estimate plasma levels of active renin, could therefore be also related to an increased conversion of inactive to active renin induced by the drug.

In conclusion, the results of the current study indicate that high dosage of Sp markedly increases plasma levels and urinary excretion of aldosterone. This effect is due to an increased aldosterone secretion since Sp also increases the metabolism of this hormone. The effect of Sp on aldosterone secretion occurs very early after administration of the drug and is probably secondary to an activation of the renin-angiotensin system. Both the increased activity of the renin-angiotensin system and aldosterone hypersecretion seems to be unrelated to alterations in Na^+ or K^+ balance.

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Resumen

Se determina la actividad renina plasmática (PRA), y la concentración plasmática de renina (PRC) y aldosterona (Aldo) en ratas tratadas con espironolactona (Sp) (Sp-rats, 20 mg/dia) y en ratas control (C-rats). Los animales se estudian a distintos días de tratamiento (1 día, 5 días y 5 semanas). Los niveles de PRA, PRC y Aldo son significativamente superiores en las Sp-rats que en las C-rats a lo largo de todo el estudio. En otros animales se determina periódicamente, durante 5 semanas, el volumen urinario, la ingesta de Na^+ y K^+, la excreción urinaria de Na^+, K^+ y de aldosterona (UAldV) y el ionograma. Posteriormente se valora la velocidad de aclaramiento de la radiactividad plasmática tras una inyección intravenosa de ^{3}H-aldosterona. No se observan diferencias significativas entre los dos grupos de animales con respecto al ionograma y balance de Na^+ y K^+. Las Sp-rats presentan una UAldV significativamente superior a las C-rats. A las 5 semanas de tratamiento la velocidad de aclaramiento de la radiactividad plas-

mática es significativamente superior en los animales tratados. Estos resultados indican que la administración de elevadas dosis de Sp estimula la liberación de renina y secreción de aldosterona, por un mecanismo no relacionado con cambios en el balance de Na^+ o K^+.

Palabras clave: Espironolactona, Metabolismo de la aldosterona, Balance de sodio.

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