# Effect of Aldosterone Antagonist on *in vitro* Production of Aldosterone and Corticosterone in Rats.* (26121)

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Several investigators(1-3) have demonstrated that spirolactones (*e.g.*, SC-8109). are antagonistic to the electrolyte-regulating effects of aldosterone at the level of the kidney tubule. It has been suggested that the spirolactones influence the pattern of renal electrolyte excretion particularly in conditions where the level of aldosterone is elevated(4,5). Agents such as amphenone inhibit aldosterone production as well as other corticoids by suppressing adrenal gland secretion(6). The spirolactones while competing with aldosterone at peripheral sites *i.e.*, the kidney tubule, do not have a direct effect upon the adrenal gland. In fact, the spirolactones have been shown to cause increased adrenal gland production of aldosterone *in vivo*(7,8). We have found increased *in vitro* production of aldosterone in adrenals taken from rats previously treated with the spirolactone, SC-8109.

*Materials and methods.* Young adult virgin male(24) and female(24) Sprague-Dawley rats averaging 210 ± 15 and 160 ± 15 g of body weight respectively were used. Twelve males and 12 females were injected with a sesame oil solution of spirolactone, SC-8109,† 10 mg/ml. Each animal received 1.5 mg/100 g of body weight, subcutaneously, once every Mon., Wed., and Fri. for 12 weeks. Control males (9 rats) and females (12 rats) were given the sesame oil vehicle (0.25 ml/100 g b.w.) on the same regimen of injection as the spirolactone. The animals were housed in air-conditioned quarters and fed a regular diet with tap water to drink *ad libitum*. At autopsy, animals were sacrificed by decapitation. Their adrenal glands were removed and trimmed free of extraneous tissue as rapidly as possible. Adrenals of 3 animals were halved and pooled for each incubation. The incubation procedure and method for extracting the steroids have been described(9). ACTH (Acton "X") in a concentration of 100 mμ/100 mg adrenal tissue was present in the incubation medium. The incubation technic was adapted from Saffran and Schally(10). The glands were preincubated for 30 min. Fresh incubation medium was added and the incubation continued for 1 hour. Total alpha-ketolic steroids in the final incubate were determined by the blue tetrazolium method of Nowaczynski *et al.*(11). Quantitative paper chromatography was used to separate the steroids. Individual steroids were determined directly by scanning the chromatograms with a recording and integrating densitometer (12). Changes in production of aldosterone and corticosterone relative to total alpha-ketolic steroids are reported here.

*Results.* No unusual changes occurred in the behavior of the animals during the course of injections. The SC-8109-treated males gained less weight than the sesame oil-injected male controls. The opposite trend was observed in the female rats (Table I). Adrenal weight of SC-8109-treated females was significantly less than the female controls ($p<0.03$).

A striking increase in relative production of aldosterone *in vitro* was found in SC-8109-treated incubated adrenals of male rats (Table I). This was accompanied by a significant reduction of corticosterone production. Female rats treated with SC-8109 also showed an increase in aldosterone with a concomitant fall in corticosterone. However, this was not as marked as in the male.

*Discussion.* The significant increase in aldosterone production ($p<0.001$) in male

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TABLE I. *In Vitro* Adrenal Production of Aldosterone and Corticosterone in Male and Female Sprague-Dawley Rats Treated with Spirolactone (SC-8109).

| Group | No. rats | Body wt, g | Adrenal wt, mg | Total steroid, μg/hr/100 mg adrenal tissue | Aldosterone (% of total steroid) | Corticosterone (% of total steroid) |
|---|---|---|---|---|---|---|
| ♂ | | | | | | |
| Sesame oil | 9 | 394 ±17* | 45.8 ± 1.9 | 11.2 ± .1 | 17.5 ± 2.1 | 52.6 ± 3.0 |
| SC-8109/sesame oil | 12 | 363 ± 8 | 42.0 ± 2.3 | 12.1 ±1.0 | 37.4 ± 1.5<br>P <.001 | 33.4 ± 1.6<br>P <.005 |
| ♀ | | | | | | |
| Sesame oil | 12 | 242 ± 6 | 65.2 ± 2.7 | 13.4 ± .5 | 13.9 ± 2.4 | 55.2 ± 2.4 |
| SC-8109/sesame oil | 12 | 260 ± 6 | 57.4 ± .5<br>P <.03 | 14.4 ± .5 | 23.1 ± 2.5<br>P <.05 | 41.4 ± 4.1<br>P <.05 |

* Stand. dev. of mean.

rats treated with SC-8109 over sesame oil-injected controls confirms what has been observed *in vivo*(7,8). The greater production of aldosterone by SC-8109-treated male adrenals compared to similarly treated females may reflect in part the greater capacity of male Sprague-Dawley rats to produce aldosterone (*in vitro*)(12). The larger renal tubular mass in males provides more opportunity for competition between aldosterone and SC-8109. This may result in increased adrenal production of aldosterone by way of feed-back mechanisms.

The significant reduction of corticosterone observed in both sexes seems to parallel the increase in aldosterone, and probably reflects changes in adrenal steroidogenic pathways which favor aldosterone production.

*Summary.* Male and female Sprague-Dawley rats were treated with the spirolactone, SC-8109, known to be an aldosterone antagonist. After 12 weeks of treatment the animals were sacrificed. Adrenal glands were incubated *in vitro* and total alpha-ketolic, as well as individual, steroids were determined. Male and female adrenal incubates from SC-8109-treated rats contained significantly more aldosterone and less corticosterone than incubates from those injected with the sesame oil vehicle. Male rats treated with SC-8109 showed particularly striking increase in aldosterone production.

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