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Mode of spirolactone action: competitive inhibition of aldosterone binding to kidney mineralocorticoid receptors*

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KAGAWA[1] obtained pharmacological evidence that the spirolactone class of aldosterone antagonists competitively interact with mineralocorticoids for receptor sites in target tissues. However, Lockett and Roberts[2] were unable to demonstrate antagonism of aldosterone-induced sodium retention by two spirolactones in adrenalectomized, hypophysectomized animals. As an alternative explanation, Lockett and Roberts suggested that spirolactones antagonize the renal actions of mineralocorticoids by changing the rate of secretion of growth hormone.

Fanestil and Edelman[3] demonstrated that kidney nuclei contain physiologically specific receptors for mineralocorticoids. This provides a method for testing the mechanism of spirolactone antagonism of aldosterone action. The results confirm Kagawa's inference by indicating that the water-soluble spirolactone, SC-14266,* competitively inhibits aldosterone interaction with renal mineralocorticoid receptors.

METHODS AND RESULTS

Extraction of unmetabolized ^{3}H-aldosterone, determination of protein and DNA, and liquid scintillation counting methods were as previously described.[3] In the first experiment, 22 adrenalectomized rats (purchased from Charles River Breeding Laboratories) were injected sc. with $28{\cdot}6 \times 10^{-11}$, $14{\cdot}3 \times 10^{-11}$ or $7{\cdot}15 \times 10^{-11}$ mole ^{3}H-aldosterone (sp. act., 35 c/m-mole; supplied by New England Nuclear Corp.). One-half of the animals simultaneously received 5×10^{-7} mole SC-14266 in 1 ml saline by a separate sc. injection. Thirty minutes later the animals were sacrificed under ether anesthesia, heparinized blood was collected by cardiac puncture and the kidneys were excised. The purified kidney nuclear fraction was isolated by centrifugation through 2·2 M sucrose as previously described in detail.[3] The amount of unmetabolized ^{3}H-aldosterone per mg DNA in the isolated nuclei was plotted against the plasma concentration of unmetabolized ^{3}H-aldosterone in double reciprocal fashion (Fig. 1). In the absence of spirolactone, the reciprocal of the nuclear aldosterone content was linearly dependent upon the reciprocal of the plasma aldosterone concentration. In the animals which received spirolactone, these relationships were again linear, but the slope of the line was markedly increased with little change in intercept value, effects that are characteristic of competitive inhibition.

In a second experiment, 10 adrenalectomized animals were injected with $2{\cdot}8 \times 10^{-9}$ mole ^{3}H-aldosterone (10 μc). Half of the animals received by separate injection $2{\cdot}8 \times 10^{-5}$ mole SC14266 (this is approximately the same ratio of aldosterone:spirolactone used in the first experiment). Thirty minutes later the kidneys were removed, homogenized and separated into purified nuclear, mitochondrial, microsomal and 100,000 *g* supernatant fractions (see ref. 3 for experimental details).

* The systematic name for SC-14266 is potassium 3-(3-oxo-17 beta-hydroxy-4,6-androstadien-17-alpha-yl) propionate.

Unmetabolized ^{3}H-aldosterone content of each fraction was determined and was expressed as moles of aldosterone per mg of protein in the fraction. The results (Table 1) show that SC14266 failed to alter plasma aldosterone concentration significantly. Although the mean aldosterone contents of the mitochondrial and microsomal fractions were lower in the SC14266-treated animals, they were not

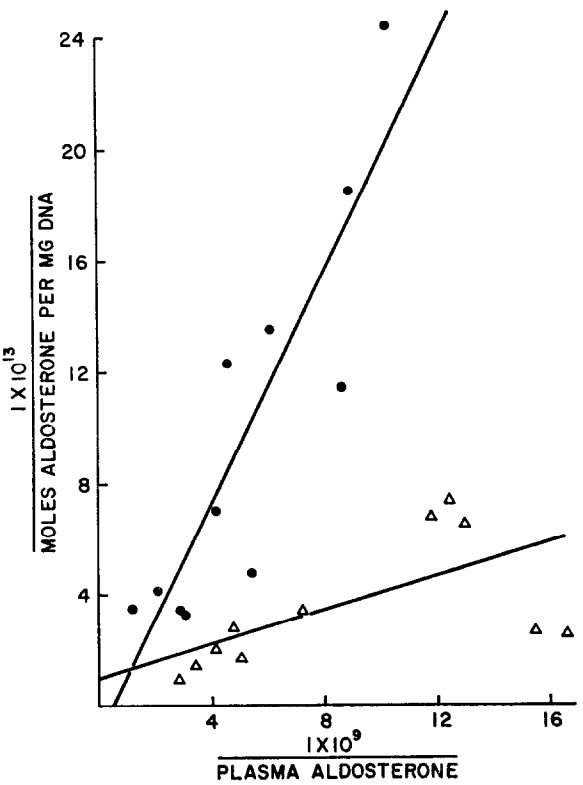

FIG. 1. Double reciprocal plot of nuclear aldosterone content vs. plasma aldosterone concentration. Plasma aldosterone was between $6{\cdot}05 \times 10^{-11}$ and $8{\cdot}7 \times 10^{-10}$ M. △, Animals received two S.C. injections, one containing ^{3}H-aldosterone and one containing isotonic saline; ●, animals received two subcutaneous injections, one containing ^{3}H-aldosterone and one containing 5×10^{-7} mole SC14266 in 1 ml isotonic saline. The lines were calculated by the method of least squares.

TABLE 1. EFFECT OF SPIROLACTONE SC14266 ON INTRACELLULAR DISTRIBUTION OF ^{3}H-ALDOSTERONE*

Fraction	Aldosterone	Aldosterone + SC14266†	P value
Plasma‡	1·24 ± 0·19	1·39 ± 0·28	> 0·50
Nuclei	5·39 ± 0·52	1·89 ± 0·54	< 0·005
Supernatant	9·39 ± 0·52	5·61 ± 0·65	< 0·005
Mitochondria	3·13 ± 0·73	2·43 ± 0·34	> 0·40
Microsomes	2·77 ± 0·63	1·67 ± 0·22	> 0·05

*Values are means ± S.E.M. of 5 animals in each group. All animals received $2{\cdot}8 \times 10^{-9}$ mole ^{3}H-aldosterone.

† These animals received, in addition, $2{\cdot}8 \times 10^{-5}$ mole SC14266.

‡ Plasma values are moles of aldosterone/l. $\times 10^{-10}$. All other values are moles of aldosterone per microgram of protein $\times 10^{-12}$.

significantly different statistically from the control animals. As expected, the quantity of aldosterone in the nuclear fraction was depressed to 35 per cent of control values by SC14266. In addition, the amount of aldosterone in the supernatant fraction was also significantly depressed to 60 per cent of control values by the SC14266. This suggests that both nuclear and supernatant cell fractions contain receptors for aldosterone and that the binding of aldosterone to the receptors in both fractions is depressed by SC14266. Indeed, Herman and Edelman[4] have recently reported the presence of a receptor for aldosterone in the "cytosol" as well as in the nuclear subcellular fractions.

In summary, after injection of aldosterone *in vivo*, uptake of the aldosterone by the nuclear fraction is depressed to 35 per cent of control and uptake in the supernatant fraction is depressed to 60 per cent of control by the simultaneous injection of a 10,000-fold molar excess of the spirolactone, SC14266. The properties of the spirolactone inhibition of aldosterone uptake by the nuclear fraction are characteristic of competitive inhibition. These results provide strong evidence that the spirolactones are aldosterone antagonists by virtue of their ability to inhibit competitively the interaction of mineralocorticoids with the physiologically important receptors for mineralocorticoids.

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