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SHORT COMMUNICATION

COMPARATIVE BIOSYNTHETIC STUDIES IN A CASE OF PRIMARY ALDOSTERONISM

VILMA DAHL*†, CARLOS M. SCATTINI and CARLOS P. LANTOS*‡
Centro de Investigaciones Endocrinológicas, Hospital de Niños; Sala 3, Hospital Rivadavia and Laboratorio de Esteroides, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

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Although the biosynthesis of steroids by adrenals and tumors from patients with primary aldosteronism [1–9] and the steroid content of such tumors [10–13] has been exhaustively studied in the past, no publication has so far analyzed the effect of ACTH *in vitro* on steroidogenic pathways of surviving aldosterone producing adenomata, as compared to those of normal adrenals.

These studies were attempted by us on tumorous and normal adrenal tissue from a patient with increased aldosterone-secretion-rates. The patient, A.V., a 32 year-old female with a history of hypertension and muscular weakness of three years of evolution, was admitted to board at the Rivadavia Hospital for clinical evaluation. Her blood pressure was 170/110 mmHg. The electrocardiogram showed a generalized flattening of the T waves and a QT interval of 0.46 s (normal value for the observed heart rate: 0.37 s). Aldosterone-secretion-rates (ASR) [14] and acid labile aldosterone excretion [15] were measured before surgery, twice under normal dietary conditions, once on the fourth day of sodium restriction (10 mequiv of sodium and 70 mequiv of potassium daily during four days) and on the first and second day of a two-day ACTH treatment (sustained release ACTH gel, 50 I.U. per day). The results of these five determinations are for ASR: 780 μg/day, 890 μg/day, 1085 μg/day, 970 μg/day and 1020 μg/day respectively and for acid labile aldosterone excretion: 42 μg/day, 45 μg/day, 51 μg/day, 48 μg/day and 57 μg/day respectively. Plasma renin levels [16]‖ were equal to 1.95 ng/ml/h under normal dietary conditions and to 11.4 ng/ml/h upon sodium restriction. The patient presented a severe hypokalemic alkalosis. After surgical removal of the tumour, ASR could be reduced to 108 μg/day, acid-labile-aldosterone excretion to 13.0 μg/day and plasma renin activity to 0.98 ng/ml/h.

The hypokalemic alkalosis was corrected, the blood pressure descended to 130/80 mmHg and the electrocardiogram was normalized. During surgery an adrenocortical tumor weighing 11 g was removed with the right adrenal. The histological examination gave the following results: adrenocortical adenoma containing large cells of clear cytoplasm; some islands containing glomerulosa-like cells.¶

Immediately after removal, the tumor and its adjacent adrenal were placed on crushed ice. Tumor samples were taken for histology and samples of adenoma and adrenal for the determination of total contents of aldosterone and corticosterone. Representative portions of both tissues were sliced into 2 mm thick slices by means of a manual microtome. These slices were assembled for incubation assays.

Aldosterone [17] and corticosterone [18] contents were determined in tissue-homogenates (30% ethanol).

Incubation assays [19, 20] were performed on four pools of normal adrenal slices and on two pools of tumor slices after 40 min preincubation, for a period of 4 h in the presence of 5.3 ng of corticosterone 1,2 ^{3}H. Two I.U. of ACTH were added to one beaker containing tumor slices and to 2 beakers containing normal adrenal slices. After incubation, tissue and media were extracted and the radioactive metabolites separated and located according to general procedures [20]. Sequential chromatographic separations are described in detail in Fig. 1. Constant $^{3}H/^{14}C$ ratios could be obtained for aldosterone and corticosterone in all cases. Unconverted tritiated corticosterone and tritiated aldosterone were calculated according to these ratios. Tritiated 18-hydroxycorticosterone was quantitated approximately, taking into account the losses of near-by running aldosterone fractions.

Radioactive steroids were obtained from New England Nuclear (Boston, Mass.) and were shown to be free of contaminants. Natural adrenocorticotrophin (500 I.U./ml) was kindly donated by Laboratorios ELEA (Argentina). According to the manufacturer, it was a mixture of equal parts of porcine and bovine ACTH. All solvents were of p.a. grade and redistilled before use.

Table 1 summarizes the results of *in vitro* parameters: hundred mg of adenomatous tissue contained over eight times more aldosterone than 100 mg of the adjacent adrenal. An increased corticosterone content can also be seen, but in this case the ratio between tumour and adrenal is much lower. During incubation, 100 mg of tumorous tissue synthesized 1460 ng of corticosterone and the same amount of adrenal tissue, an average of 848 ng of corticosterone. After incubation, the yields of unconverted radioactive precursor (corticosterone) were 75% (average) for the adrenal and 31% for the tumor. Concomitantly the tumor synthesized 39% of aldosterone and 25% of 18-hydroxycorticosterone while the adrenal synthesized only one-eighth of this amount of aldosterone and even less 18-hydroxycorticosterone.

The table also shows the effect of ACTH on the endogenous production of corticosterone and conversions of tritiated corticosterone to metabolites by both tissues. The trophin increased the endogenous production of corticosterone both in the tumor and the adjacent gland. However, while not affecting the conversion of tritiated corticoster-

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* Career Investigator of the National Research Council of Argentina.

† Reprint requests should be sent to Dr. Vilma Dahl, Centro de Investigaciones Endocrinológicas, Hospital de Niños, Gallo 1330, Buenos Aires, Argentina.

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‖ Plasma renin activity was determined by Dr. Nidia Basso (Centro de Investigaciones Cardiológicas. Buenos Aires. Argentina).

¶ The histological examination was performed by Dr. Ricardo Colillas in the laboratory of Pathological Anatomy of the Rivadavia Hospital.

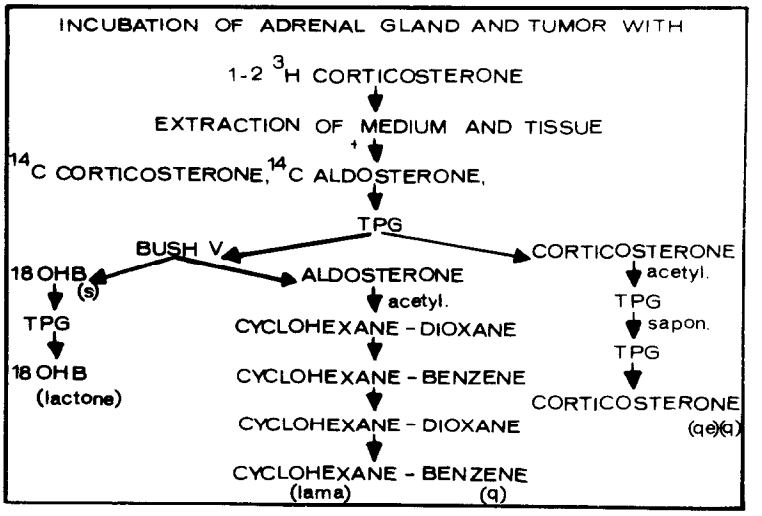

Fig. 1. Analytical procedures. Abbreviations—acetyl: acetylated; sapon: saponified; (q): radioactive fraction quantitated; (qe): endogenous steroid quantitated; (s): radioactive fraction semiquantitated i.e. corrected for losses according to those experienced by nearby running fractions containing ^{14}C tracers; lama: lactone of aldosterone monoacetate; TPG: toluene–propylene–glycol; Bush V: Bush B5. All separation media refer to paper-chromatographic systems. The arrow from "TPG" to "Bush V" indicates that radioactive material at the origin of TPG chromatograms had been transferred to Bush V.

Table 1.

	Aldosterone content ng/100 mg	Corticosterone content ng/100 mg	Endogenous corticosterone ng/100 mg	Corticosterone 1,2 ^3H %	Aldosterone 1,2 ^3H %	18-OHB 1,2 ^3H %
Tumor control	101	214	1460	31.0	39.0	25.8
Tumor + ACTH			3911	42	26.6	28.7
Adrenal control	12	55	900	80.0	5.0	2.0
			757	70.1	5.7	2.4
Adrenal + ACTH	–		1655	74.8	5.3	3.9
			1550	72.8	5.3	4.7

Results of *in vitro* parameters. Aldosterone content, corticosterone content: tissue corticosterone and aldosterone content. Endogenous corticosterone: endogenous corticosterone produced during incubation. Corticosterone 1,2 ^3H: unconverted radioactive corticosterone. Aldosterone 1,2 ^3H: converted aldosterone. 18-OHB 1,2 ^3H: converted 18 hydroxycorticosterone. Each value corresponds to one determination.

one to metabolites by normal adrenal tissue, ACTH, as judged by the results of single incubation samples, seems to inhibit the transformation of the radioactive precursor into aldosterone by tumor slices.

Since the tumor contains and synthesizes approx. eight times more aldosterone than the normal adrenal, and since the ratio between the patient's aldosterone secretion rates before and after removal of the tumor is also close to eight, it is reasonable to believe that the latter is responsible for the increased mineralocorticoid secretion observed *in vivo* and that *in vitro* parameters related to aldosterone are in this case representative of aldosterone production in the living organism.

Comparative studies on the *in vitro* effect of ACTH could be performed on duplicate controls but only on single tumor samples. The results suggested that ACTH exerts an inhibitory effect on the formation of aldosterone by the tumor while not affecting the production of this steroid by the normal adrenal. It seems improbable that this inhibitory effect of the trophin in the tumor should be due to competition of the increased endogenous corticosterone pool for aldosterone synthesizing enzymes: for one thing, corticosterone pools are augmented more or less to the same extent by ACTH in incubation vessels containing normal and tumorous slices. On the other hand, the higher aldosterone content of the adenoma suggests that the amount of aldosterone synthesizing enzymes therein is not limiting.

These observations were performed on the limited amount of material available from tumor and adrenal of one single case of a Conn syndrome. This disease being relatively infrequent the findings are given as a preliminary report which might pave the way towards further comparative regulatory studies in adrenocortical diseases. Such studies are presently undertaken in our laboratories.

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