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Research Article

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The specificity of the sodium depletion stimulus on the conversion of corticosterone to aldosterone was established by comparing two other mitochondrial enzymes from glomerulosa cell mitochondria. Succinic dehydrogenase and 11 β-hydroxylase were measured in normal and sodium-depleted rats and no difference in activity of either enzyme was found.

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Abstract. The effect of various factors on the conversion of corticosterone to aldosterone was studied in an isolated mitochondrial system from rat adrenal glands. The adrenal mitochondrial fraction from rats on a low sodium diet has a greater capacity for converting corticosterone to aldosterone than mitochondria from rats fed a normal diet. After 1 day on a low sodium diet the amount converted was 162% and after the 2nd and 4th day the amounts converted were 239 and 242%, respectively, compared to a value of 100% for the control rats. Sodium and(or) potassium added in vitro did not affect the conversion of corticosterone to aldosterone.

The specificity of the sodium depletion stimulus on the conversion of corticosterone to aldosterone was established by comparing two other mitochondrial enzymes from glomerulosa cell mitochondria. Succinic dehydrogenase and 11 β-hydroxylase were measured in normal and sodium-depleted rats and no difference in activity of either enzyme was found.

The data are consistent with the view that sodium depletion stimulates the last step in aldosterone biosynthesis by causing a specific enzymatic change in adrenal mitochondria.

Introduction

Sodium depletion is a potent stimulator of aldosterone secretion. This stimulation appears to be through a humoral mechanism. Although there are many studies on the regulation of aldosterone secretion, only a few attempt to define the site in the biosynthetic pathway that is stimulated by sodium depletion (1–5). One of the chief problems in such a study is that many of the steps in the aldosterone biosynthetic pathways are common to biosynthesis of other corticoids. Since these other corticoids and their precursors are pro-

duced at a much greater rate than aldosterone, any changes in the aldosterone pathway may be masked when the whole adrenal gland is studied in vivo or in vitro.

The biosynthesis of aldosterone occurs in the zona glomerulosa of the adrenal gland and little, if any, takes place in the fasciculata-reticularis zones (6). The only step that is unique in the biosynthesis is the conversion of corticosterone to aldosterone, which occurs in the mitochondrial fraction (7–9). The steps involved are presumably corticosterone → 18-hydroxycorticosterone → aldosterone. Only the production of aldosterone is specific for the zona glomerulosa. Some 18-hydroxycorticosterone is synthesized in the zona fasciculata-reticularis as well, but the quantity appears to vary with different species (3, 7).

The present experiments were designed to determine whether in vivo sodium depletion stimulated the last steps in aldosterone biosynthesis: conversion of corticosterone to aldosterone. The rat was selected as the experimental animal since short-

term sodium depletion can be easily induced and the zona glomerulosa can be readily separated from the inner zones (10). The use of the mitochondrial fraction eliminated the influence of corticosterone precursor pools on the conversion of corticosterone to aldosterone. The results indicate that short-term sodium depletion stimulates the last steps in aldosterone biosynthesis by producing a specific enzymatic change in adrenal glomerulosa cell mitochondria.

Methods

Male albino Sprague-Dawley rats, weighing 150–200 g were used in this study. The rats were fed a specially prepared diet with either a normal or low sodium content (11). The animals were killed by decapitation and the adrenal glands immediately removed, freed of fat, and homogenized in 0.25 M sucrose. When only capsular tissue was used, it was obtained as described by Giroud et al. (10) and special care was taken to eliminate the inner zones. The capsules were then homogenized. The mitochondrial fraction was obtained by the method of Schneider (12). Protein nitrogen was determined by the Lowry method (13) and succinic dehydrogenase according to Slater and Bonner (14).

The incubation media, unless otherwise indicated, contained 40 mM potassium phosphate buffer pH 7.4, 5 mM $MgCl_2$, 10 mM sodium fumarate, 0.25 mM NADP, 4.5 mM glucose-6-phosphate, and 1 U of glucose-6-phosphate dehydrogenase in a final volume of 2 ml. 20 µg of corticosterone-4-^{14}C was added to each flask which contained about 0.25 mg of mitochondrial protein nitrogen. When the incubation was carried out in 20 mM Tris-HCl buffer pH 7.4, the same cofactors that were used in the phosphate buffer were added to the incubation media. In the Krebs-Ringer bicarbonate buffer only fumarate and the NADPH generating system were added in the concentrations indicated above. The incubation was carried out for 30 min at 37°C in air in a Dubnoff incubator; when a longer incubation time was used, the time is indicated in the results.

When 11β-hydroxylase activity was measured, 100 µg of deoxycorticosterone (DOC) was added instead of corticosterone and the incubation was carried out for 15 min. The amount of DOC converted to corticosterone was used as an index of 11β-hydroxylase activity.

Extraction and identification of the steroids. Tracer amounts of aldosterone-1,2-^{3}H were added to correct for losses in purification. The samples were extracted with 10 volumes of methylene chloride. The extracts were washed sequentially with 1/10 volume 0.05 N NaOH, 0.1 M acetic acid, and distilled water, and evaporated in vacuo. Stable aldosterone and cortisol were added and the dried residue was applied to a paper chromatogram and run in the formamide-chloroform system (15). The aldosterone and corticosterone areas were located by their absorption of ultraviolet light. The 18-hydroxycorticosterone area was located by radiochromatogram scanning

and its R_s (cortisol). Identification of this zone was accomplished in certain samples as previously described (7).

An aliquot of the aldosterone area from the first chromatogram was taken for counting and the remainder was rechromatographed in the Bush B_5 system, eluted, and acetylated with pyridine–acetic anhydride 1:2 for 24 hr. The acetylated derivative was chromatographed in the cyclohexane-benzene-methanol-water 100:50:100:25 and cyclohexane-dioxane-methanol-water 100:100:50:25 systems and the ^{3}H/^{14}C ratio was measured.

Aldosterone accounted for 89.2% ± 3.7 (mean and standard deviation of 11 experiments) of the radioactivity in the aldosterone area after the first chromatographic system. Since good purification was obtained from the first chromatogram system, these counts were used in most of the calculation. Each time conditions were changed, the diacetate was again made to check the aldosterone purity. In each instance there was no evidence of contamination.

The per cent of corticosterone-4-^{14}C converted to 18-hydroxycorticosterone and aldosterone was calculated. Aldosterone-^{14}C counts were corrected for losses by ^{3}H tracer. No correction was made in the 18-hydroxycorticosterone samples. The aldosterone production in the samples incubated with stable DOC was measured by the double isotope technique (16). Corticosterone was measured by the acid fluorescence method (17). Radioactivity was measured with a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Corticosterone-4-^{14}C and D-aldosterone-1,2-^{3}H were obtained from New England Nuclear Corp. (Boston, Mass.) and purified by further paper chromatography.

Results

The conversion of corticosterone to aldosterone by the different adrenal cellular fractions is shown in Fig. 1. Whole homogenate, nuclear, mitochondrial, microsomal, and soluble fractions obtained from 100 mg of adrenal tissue were incubated in the presence of 40 µg of corticosterone-4-^{14}C. Combinations of soluble fraction plus mitochondrial, and soluble fraction with microsomal fractions were also tested. As shown in Fig. 1, all the conversion activity is present in the mitochondrial fraction. The amount of aldosterone converted from corticosterone-4-^{14}C from this fraction was 1.96% (0.78 µg of aldosterone per 100 mg of tissue). No further increase of activity of the mitochondrial fraction was observed when the soluble fraction was added to the incubation media (0.80 µg of aldosterone per 100 mg of tissue). The small amount of conversion in the nuclear and microsomal fractions is probably due to mitochondrial contamination of these fractions.

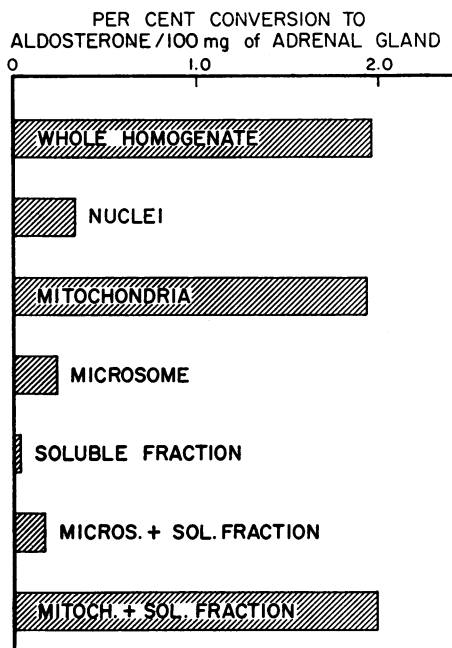The conversion of corticosterone to aldosterone

PER CENT CONVERSION TO
ALDOSTERONE / 100 mg of ADRENAL GLAND

FIG. 1. CONVERSION OF CORTICOSTERONE-4-^{14}C TO ALDOS-
TERONE BY DIFFERENT CELL FRACTIONS FROM THE WHOLE
ADRENAL GLAND. Incubation conditions were as noted un-
der Methods. 40 μg of corticosterone-4-^{14}C was used per
flask. The incubation was carried out for 1 hr. Results
are the mean of two experiments and are expressed as per
cent corticosterone converted per 100 mg adrenal gland.

by adrenal capsular mitochondria was compared
with that of the inner zones in two experiments.
The capsular mitochondria converted 11.6% of the
corticosterone-4-^{14}C added, while only 0.2% was
converted by the fasciculata-reticularis mitochon-
dria. This relationship was also observed in the
sodium-depleted rats, where a conversion of 22.6%
was found in the capsular mitochondria, and only
0.3% for the inner zones. These values are ex-
pressed as per cent of corticosterone converted to
aldosterone per 0.25 mg of mitochondrial nitrogen.

The effect of a low sodium diet on the conver-
sion of corticosterone to aldosterone by adrenal
mitochondria from the whole gland is shown in
Fig. 2. The per cent converted per milligram of
protein nitrogen by the mitochondrial fraction of
rats maintained on a normal sodium diet (control
rats) is expressed as 100% and the per cent con-
verted by the sodium-depleted rats as per cent of
controls. After 1 day on a low sodium diet there
is an increase of 162% in the per cent of corti-
costerone converted to aldosterone, and this in-

creases to 239 and 242% by the 2nd and 4th day
of sodium depletion, respectively.

To determine if the supernatant fraction ob-
tained from sodium-depleted rats might have had
an effect on mitochondrial enzymatic activity, we
performed two experiments in which the conver-
sion of corticosterone to aldosterone was measured
in the mitochondrial fraction from sodium-depleted
rats with and without the addition of the super-
natant fraction. The mitochondrial fraction alone
had a conversion rate 264% greater than that of
the control, while the mitochondrial fraction plus
the supernatant fraction was also 260% greater,
indicating that the supernatant fraction from so-
dium-depleted rats did not enhance the conversion
of corticosterone to aldosterone. Moreover, the
increased conversion by mitochondria from so-
dium-depleted rats is present regardless of whether
furmarate, malate, or succinate was added as a
source of substrate for the respiratory chain.

Furthermore, addition of NADPH to the in-
cubation media was not necessary to show this dif-
ference between mitochondria from control or
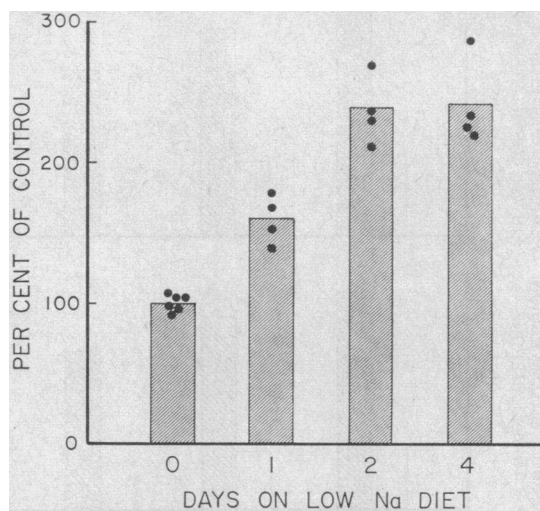sodium-depleted rats. In three experiments in

FIG. 2. CONVERSION OF CORTICOSTERONE-4-^{14}C TO ALDOS-
TERONE BY THE WHOLE ADRENAL MITOCHONDRIAL FRACTION
OF RATS AFTER 0 (CONTROL), 1, 2, AND 4 DAYS OF A LOW
SODIUM DIET. The results are expressed as per cent of
the mean control value. The mean control value was
7.3% conversion per milligram of mitochondrial protein
nitrogen; this value is expressed as 100%. The range of
conversion by the control mitochondria was between 6.4
and 7.9%. Each dot represents the result of an indi-
vidual experiment.

which no NADPH was added to the media and
malate was used instead of fumarate, 1 day of
sodium depletion resulted in 173% greater conver-
sion, and 2 days in 202% greater conversion than
the control mitochondria. Therefore, it did not
appear that sodium depletion merely altered the
permeability of the mitochondria to allow more
NADPH to enter.

The conversion of corticosterone to 18-hydroxy-
corticosterone was also studied. As shown in
Fig. 3, the mitochondrial fraction of whole glands
obtained from sodium-depleted rats demonstrated
an increase in the ability to convert corticosterone
to 18-hydroxycorticosterone, which paralleled the
conversion of corticosterone to aldosterone. Al-
though no correction was made for losses of 18-
hydroxycorticosterone in these samples, this in-
crease was constant in all experiments.

The effect of different ionic media on the con-
version of corticosterone to 18-hydroxycorticos-
terone to aldosterone is also shown in Fig. 3.
Krebs-Ringer bicarbonate, potassium phosphate,
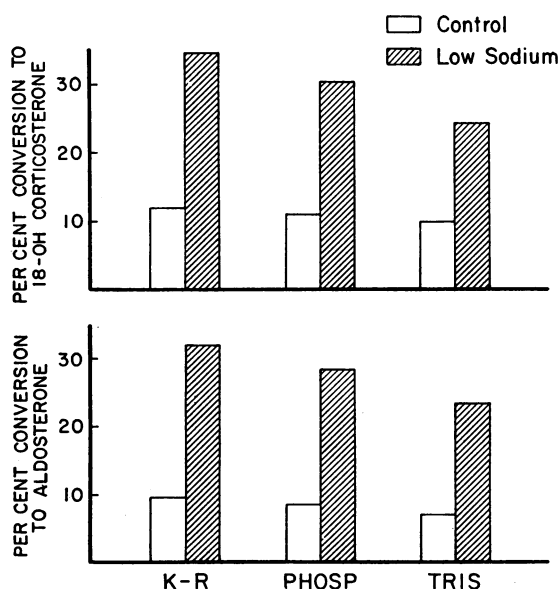and Tris buffers were used. The different media

FIG. 3. EFFECT OF DIFFERENT BUFFERS ON THE CON-
VERSION OF CORTICOSTERONE-4-^{14}C TO ALDOSTERONE AND 18-
HYDROXYCORTICOSTERONE BY CONTROL AND LOW SODIUM RAT
ADRENAL MITOCHONDRIA OBTAINED FROM THE WHOLE GLAND.
K-R, Krebs-Ringer bicarbonate; Phosp, potassium phos-
phate buffer; Tris, 20 mM Tris buffer pH 7.4. The re-
sults are expressed as per cent corticosterone converted
per milligram of mitochondrial protein nitrogen. The in-
cubation was carried out for 1 hr.

TABLE I

Effect of different buffers

| Experi-ment | Corticosterone converted to aldosterone | | | |
	Sodium phosphate	Potassium phosphate	Tris chloride	Krebs-Ringer
	%	%	%	%
9	6.7	5.2	—	—
21	7.2	7.0	5.7	8.3
24	—	7.5	5.5	—
27	—	8.8	7.1	9.8

Effect of different buffers on the conversion of corticos-
terone to aldosterone by whole adrenal mitochondria
from rats fed a normal sodium diet. Results are expressed
as per cent converted per milligram of mitochondrial
nitrogen.

did not affect the enhanced conversion rate by mito-
chondria of sodium-depleted rats, although in the
Tris buffer the absolute amount converted was
smaller for both the control and sodium-depleted
groups.

Furthermore, comparison of the effect of buf-
fers of different ionic composition (sodium phos-
phate, potassium phosphate, Krebs-Ringer bicar-
bonate, Tris, and Tris plus addition of sodium
and potassium chloride) on the conversion of corti-
costerone to aldosterone by the control rats mito-
chondria showed no striking difference despite
marked variation in the sodium and potassium
concentrations (Table I). In one experiment
(not included in Table I) different amounts of
sodium and (or) potassium were added to the
Tris buffer, from 0 to 120 millimoles/liter. Vary-
ing the concentrations of the sodium and (or)
potassium had no effect on the conversion of
corticosterone to aldosterone.

The effect of substrate concentration on the in-
creased conversion of corticosterone to aldosterone
by the sodium-depleted rats was tested using 1, 5,
10, 20, 40, and 100 μg of corticosterone per flask.
As is shown in Fig. 4, the enhanced conversion
by the 4 day low sodium rat adrenal mitochondria
was present throughout the substrate concentra-
tion range studied.

Further studies were designed to measure the
specificity of the stimulus in the conversion of
corticosterone to aldosterone by the low sodium
rat mitochondria. Two other mitochondrial en-
zymes were simultaneously measured and com-
pared in both groups. The enzymes selected were
succinic dehydrogenase, a typical mitochondrial
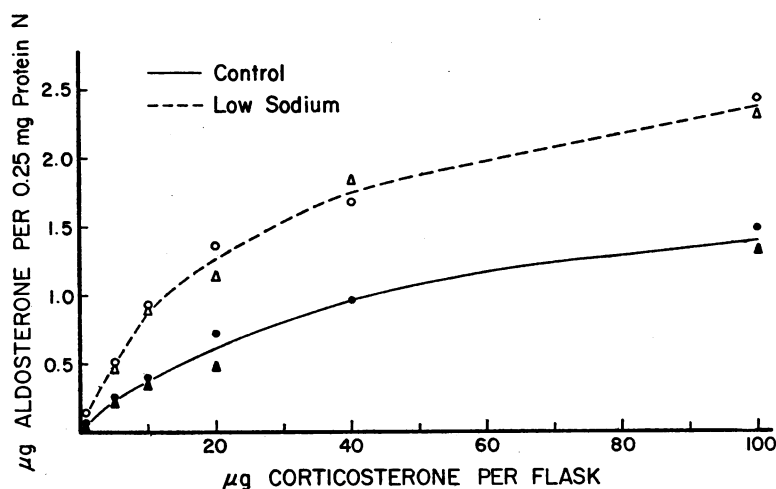enzyme, and 11β-hydroxylase. 11β-Hydroxylase

FIG. 4. EFFECT OF SUBSTRATE CONCENTRATION ON THE AMOUNT OF AL-
DOSTERONE PRODUCED BY MITOCHONDRIA FROM CONTROL (SOLID LINE) AND
SODIUM-DEPLETED RATS (DASHED LINE). The results from each of four sepa-
rate experiments are plotted individually; the open circles and triangles rep-
resent sodium-depleted rats and the closed circles and triangles represent
normal rats. Between 0.25 and 0.28 mg of mitochondrial protein nitrogen
were added to each flask. The aldosterone results are corrected to 0.25 mg
of protein nitrogen.

is involved in the conversion of DOC to corticos-
terone, the step immediately preceding the last
step in aldosterone biosynthesis. For this study it
was necessary to separate the glomerulosa zone
from inner zones since most of the mitochondria
from the whole gland comes from fasciculata-
reticularis cells. The capsules of 20–30 rats, con-
taining mostly glomerulosa cells, were used in each
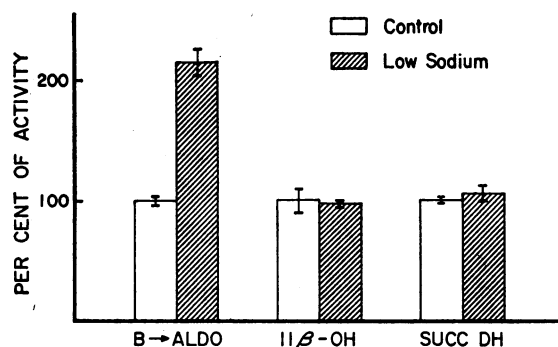experiment.

FIG. 5. COMPARISON OF ADRENAL CAPSULAR MITOCHON-
DRIA ENZYMES FROM CONTROL AND SODIUM-DEPLETED RATS.
The values of the control rats are expressed as 100% and
those of the low sodium rats as per cent of the controls.
Mean values and SEM are shown. $B \rightarrow Aldo$, conversion
of corticosterone to aldosterone; 11β-OH, 11β-hydroxy-
lase activity; *Succ DH*, succinic dehydrogenase activity.

Results of the succinic dehydrogenase activity,
11β-hydroxylase, and conversion of corticosterone
to aldosterone are shown in Fig. 5. The results
per milligram of mitochondrial protein nitrogen
from the 4-day depleted rats were compared with
those from rats on a normal sodium intake. The
values from the control rats were expressed as
100%. The mean and standard error of the mean
of three experiments are given. Succinic dehy-
drogenase and 11β-hydroxylase activities were of
the same order of magnitude for both groups, but
a marked increase of 239% in the conversion of
corticosterone to aldosterone was present in the
4-day low sodium groups.

TABLE II

*Conversion of DOC to corticosterone and aldosterone
by adrenal capsular mitochondria*

Experi-ment	Diet	Corticosterone		Aldosterone	
		μg*	% change	μg*	% change
60	Normal	100		1.0	
	Low Na	113	+13	3.4	+340
64	Normal	77		1.0	
	Low Na	85	+10	2.8	+280

15 min incubation.
* Micrograms produced per milligram of mitochondrial
protein nitrogen.

In two experiments DOC was used instead of corticosterone to determine its conversion to aldosterone since DOC is the precursor presented to the mitochondria; these experiments resemble more closely what occurs in vivo. The results are shown in Table III. DOC is rapidly converted to corticosterone by capsule mitochondria of the control and sodium-depleted rats. There was little difference between these groups. A marked increase in the conversion of DOC to aldosterone was present in the low sodium group. This is further evidence of an increase in the rate of the last biosynthetic steps, conversion of corticosterone to aldosterone, and makes it an unlikely possibility that the effect of sodium depletion is merely the result of increased permeability of the mitochondria to corticosterone.

Discussion

The major pathway for aldosterone biosynthesis appears to be cholesterol → pregnenolone → progesterone → DOC → B → 18-hydroxycorticosterone → aldosterone. This synthesis takes place in the zona glomerulosa cells of the adrenal gland. The conversion of corticosterone to aldosterone occurs in the mitochondrial fraction of the cells.

There are four possible ways by which sodium depletion may stimulate aldosterone biosynthesis: (a) stimulate the early steps in the pathway, thus delivering more corticosterone to the mitochondrial site for conversion to aldosterone; (b) stimulate the last steps, conversion of corticosterone to aldosterone; (c) produce hypertrophy or hyperplasia of the zona glomerulosa cells, and therefore increase the number of biosynthetic units (the increased aldosterone secretion would result from more units and not from an increased rate of any specific step); and (d) Stimulate by some combination of these three mechanisms.

One of the problems in studying the first steps, conversion of cholesterol to corticosterone, in the whole gland is that most of the corticosterone and its precursors are made in the fasciculata-reticularis zones. Therefore, any changes in the rate of conversion of cholesterol to corticosterone by the glomerulosa cells would be difficult to detect. Bledsoe, Island, and Liddle (4) attempted to minimize any contribution to corticosterone secretion from the fasciculata-reticularis zones by suppression of ACTH secretion with dexamethasone ther-

apy. They found that sodium depletion in man stimulated corticosterone and aldosterone secretion several fold and interpreted these data to indicate that the early steps were increased. It is possible that the increased corticosterone secretion in man was a direct effect of angiotensin on the zona fasciculata cells. The argument against this possibility was that cortisol secretion was not increased, and hence there was no stimulation of the zona fasciculata. This argument presumes that the pathway from progesterone to corticosterone is under the same control as progesterone to cortisol. If corticosterone production in the zona fasciculata were preferentially stimulated by angiotensin, this reasoning would be invalid. The evidence concerning this point is contradictory. Incubation of angiotensin with beef adrenal slices consisting chiefly of fasciculata cells produced a threefold increase in corticosterone production and only a twofold increase in cortisol production (1). In the hypophysectomized dog, however, infusions of angiotensin stimulate corticosterone and cortisol secretion to the same extent (18). Another possible criticism is that their subjects were sodium depleted by administration of diuretics and a sodium-deficient diet for approximately 1–2 wk. Hypertrophy of the zona glomerulosa may have occurred and the increased number of biosynthetic units may have contributed to the increased corticosterone and aldosterone production.

Baniukiewicz et al. have shown in vivo that the conversion to aldosterone of tritiated corticosterone infused into sheep adrenal transplants was increased by mild sodium depletion, a finding that suggests an increase in the last steps. However, in severely sodium-depleted sheep, the conversion of corticosterone to aldosterone was decreased (3). As the authors pointed out, this inhibition of the conversion of corticosterone to aldosterone when high aldosterone secretion was present could be due to a simultaneous stimulation of another pathway, or inhibition of the transport of the radioactive corticosterone into the mitochondrial active site; but no evidence is available to support either possibility.

In addition these authors, studying hypophysectomized rats, found that sodium loading decreased the in vitro synthesis of corticosterone as well as the conversion of corticosterone to aldosterone. This effect on corticosterone synthesis was notice-

able only during the first 30 min of incubation and not in the subsequent periods.

Another study of the biosynthetic steps stimulated by sodium depletion was carried out in hypophysectomized dogs. Sodium depletion increased corticosterone, cortisol, and aldosterone secretion (2). Since the increase in corticosterone secretion was greater than that of cortisol, the authors concluded that sodium depletion stimulated the early steps. The same argument presented in relationship to the human studies holds here as well. It is of interest that these results are at variance with the previous studies which showed corticosterone and coritsol to be stimulated to the same degree by angiotensin infusions. These authors (2) also showed that the conversion of corticosterone to aldosterone by dog adrenal gland slices was increased by sodium depletion, indicating stimulation of the last steps. However, since intraadrenal pool size of corticosterone was not measured, changes in pool size might account for the increase. In fact, the corticosterone production of the adrenal slices from sodium-depleted dogs was one-half that of slices from normal dogs.

Our studies were designed to avoid these limitations: by studying short-term sodium depletion in adult rats we minimized cell hypertrophy or hyperplasia, and by using an isolated mitochondrial fraction we minimized the influences of pool size of corticosterone and its precursors on the conversion of corticosterone to aldosterone. Also, the zona glomerulosa cells can be readily separated from the inner zones in the rat. The studies showed that the mitochondrial fraction from rats fed a low sodium diet for 1, 2, or 4 days has an increased rate of conversion of corticosterone to aldosterone. This increased rate of conversion is present throughout a wide range of substrate corticosterone concentration and appears to be independent of the sodium and potassium composition, NADPH, or a specific Krebs cycle intermediate in the incubation media. Cell hypertrophy or hyperplasia, which result in more mitochondria, seems an unlikely explanation since histological studies of these adrenals show little, if any, change in zona glomerulosa cell width. In the experiments with adrenal capsules, the failure to find an increase in the activity of two other mitochondrial enzymes, 11β-hydroxylase and succinic dehydrogenase, indicates that there was no increase in the number

of mitochondria but rather a specific change in mitochondrial enzyme activity: conversion of corticosterone to aldosterone. This conclusion is limited by the fact that the glomerulosa zone of capsular tissue is contaminated by fasciculata cells. Histological studies of the capsular tissue suggest that there are from one-half to an equal number of fasciculata cells as there are glomerulosa cells. However, it would require twice as much contamination to mask any change in succinic dehydrogenase and 11β-hydroxylase activity completely.

Since there is no difference in the conversion of DOC to corticosterone by the mitochondria from normal or sodium-depleted rats, it appears unlikely that sodium depletion changes the permeability of the mitochondria to allow more steroid precursor to reach the enzymatic site for conversion to aldosterone. Similar results were obtained by McCarthy, Peron, and Guerra, in long-term sodium-depleted rats (19). The fact that 11β-hydroxylase activity is unchanged suggests that there is no stimulation of the hydroxylation electron transfer chain or the respiratory electron transfer chain. Stimulation of hydroxylation by addition of maximal quantities of certain Krebs' cycle intermediates to the incubation media could not abolish the difference between the mitochondria from normal and sodium-depleted rats. It appears, therefore, that short-term sodium depletion stimulates the last steps in aldosterone biosynthesis through a specific enzymatic change in zona glomerulosa cell mitochondria. With prolonged sodium depletion an increased number of biosynthetic units develop by hypertrophy and hyperplasia of glomerulosa cells and this must also play a role. Stimulation of the early steps by delivering more corticosterone to the mitochondria can also lead to increased aldosterone secretion, as was shown in the substrate studies. These present studies have no bearing on whether early steps were stimulated, although preliminary experiments with capsular homogenates from 2-day sodium-depleted rats did not reveal increased corticosterone production (20).

It is of interest that neither the sodium nor the potassium concentration of the incubation media influenced the mitochondrial conversion of corticosterone to aldosterone, yet in vivo, the serum sodium and potassium concentrations can alter aldosterone secretion. These results may indicate

that these ions influence aldosterone secretion by affecting an earlier step in the pathway.

The mechanism by which sodium depletion stimulates the enzymatic activity of the last steps is not clear. Presumably, sodium depletion stimulates release of a humoral factor which must alter the activity of a specific mitochondrial enzyme either through stimulating synthesis of a new enzyme or increasing enzyme activity. The reactions that take place in these series of events are completely unknown. In the rat, there is conflicting evidence as to whether angiotensin is the humoral factor, since infusions of angiotensin have little effect on aldosterone secretion (21). Moreover, Müller (5) could not show a stimulation with angiotensin of the conversion of corticosterone to aldosterone by rat adrenal quarters in vitro. With beef adrenal slices, Kaplan and Bartter (1) were able to show a stimulation by angiotensin of the conversion of cholesterol to aldosterone but not corticosterone to aldosterone. Since sodium depletion stimulates the last steps, these studies suggest that a mechanism other than angiotensin is responsible for the increased conversion of corticosterone to aldosterone in the rat.

Acknowledgment

References

1. Kaplan, N. M., and F. C. Bartter. 1962. The effect of ACTH, renin, angiotensin II, and various precursors on biosynthesis of aldosterone by adrenal slices. *J. Clin. Invest.* **41**: 715.

2. Davis, W. W., L. R. Burwell, G. Kelley, A. G. T. Casper, and F. C. Bartter. 1966. Studies of the loci of stimulation of aldosterone biosynthesis during sodium depletion. *Biochem. Biophys. Res. Commun.* **22**: 218.

3. Baniukiewicz, S., A. Brodie, C. Flood, M. Motta, M. Okamoto, J. F. Tait, S. A. S. Tait, J. R. Blair-West, J. P. Coghlan, D. A. Denton, J. R. Goding, B. A. Scoggins, E. M. Wintour, and R. D. Wright. Adrenal biosynthesis of steroids *in vitro* and *in vivo* using continuous superfusion and infusion procedures. *In* Functions of the Adrenal Cortex. K. W. McKerns, editor. In press.

4. Bledsoe, T., D. P. Island, and G. W. Liddle. Studies of the mechanism through which sodium depletion increases aldosterone biosynthesis in man. 1966. *J. Clin. Invest.* **45**: 524.

5. Müller, J. 1966. Aldosterone stimulation in vitro: III. Site of action of different aldosterone-stimu-

lating substances on steroid biosynthesis. *Acta Endocrol.* **52**: 515.

6. Stachenko, J., and C. J. P. Giroud. 1964. Further observations on the functional zonation adrenal cortex. *Can. J. Biochem. Physiol.* **42**: 1777.

7. Marusic, E. T., and P. J. Mulrow. 1967. *In vitro* conversion of corticosterone-4-^{14}C to 18-hydroxycorticosterone by zona fasciculata-reticularis of beef adrenal. *Endocrinology.* **80**: 214.

8. Psychoyos, S., H. H. Tallan, and P. Greengard. 1966. Aldosterone synthesis by adrenal mitochondria. *J. Biol. Chem.* **241**: 2949.

9. Raman, P. B., D. C. Sharma, and R. I. Dorfman. 1966. Aldosterone biosynthesis in vitro. *Biochemistry.* **5**: 1795.

10. Giroud, C. J. P., J. Stachenko, and E. H. Venning. 1956. Secretion of aldosterone by the zona glomerulosa of rat adrenal glands incubated in vitro. *Proc. Soc. Exptl. Biol. Med.* **92**: 154.

11. Manitius, A., H. Levitin, D. Beck, and F. H. Epstein. 1960. On the mechanism of impairment of renal concentrating ability in potassium deficiency. *J. Clin. Invest.* **39**: 684.

12. Schneider, W. C. 1957. Methods for the isolation of particulate components of the cell. *In* Manometric Techniques. W. N. Umbreit, R. H. Burris, and J. F. Stauffer, editors. Burgess Publishing Co., Minneapolis. 3rd edition. 188.

13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1957. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265.

14. Slater, E. C., and W. D. Bonner. 1952. The effect of fluoride on the succinic oxidase system. *Biochem. J.* **52**: 185.

15. Neher, R. 1964. Steroid chromotography. Elsevier Publishing Co., Amsterdam. 2nd edition. 181.

16. Kliman, B., and R. E. Peterson. 1960. Double isotope derivative assay of aldosterone in biological extracts. *J. Biol. Chem.* **235**: 1639.

17. Silber, R. H., R. D. Busch, and R. Oslapas. 1958. Practical procedure for estimation of corticosterone and hydrocortisone. *Clin. Chem.* **4**: 278.

18. Slater, J. D. H., B. H. Barbour, H. H. Henderson, A. G. T. Casper, and F. C. Bartter. 1963. Influence of the pituitary and the renin-angiotensin system on the secretion of aldosterone, cortisol, and corticosterone. *J. Clin. Invest.* **42**: 1504.

19. McCarthy, J., F. G. Peron, and F. C. Guerra. 1965. Adrenal corticosteroidogenesis by mitochondria from rats given normal and sodium deplete diets. *Excerpta Med. Internat. Congr. Ser.* **99**. E 82.

20. Marusic, E. T., and P. J. Mulrow. 1967. Stimulation of aldosterone biosynthesis in adrenal mitochondria by sodium depletion. *J. Clin. Invest.* **46**: 1092.

21. Marieb, N. J., and P. J. Mulrow. 1965. Role of the renin-angiotensin system in the regulation of aldosterone secretion in the rat. *Endocrinology.* **76**: 657.