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MULTIFACTORIAL REGULATION OF THE FINAL STEPS OF ALDOSTERONE BIOSYNTHESIS IN THE RAT*

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SUMMARY

Several known stimulators, including angiotensin II, potassium ions, serotonin and ACTH, act directly on the zona glomerulosa of the rat adrenal cortex during short-term incubation experiments. They rapidly enhance an early biosynthetic step involved in the conversion of cholesterol to pregnenolone. The steroidogenic response elicited by these agents depends on the activity of the enzymes necessary for the final stages of aldosterone biosynthesis, in particular the 18-hydroxylation of corticosterone. The activity of these enzymes is in turn regulated by unknown mediators, which act in response to alterations in sodium and potassium balance. According to indirect evidence, the serum potassium appears to be an important but not the only regulator. Angiotensin II and serotonin may also have a long-term stimulating effect on the final steps of aldosterone biosynthesis. By contrast, treatment of rats with ACTH for a few days causes a marked decrease in the conversion of tritiated corticosterone to 18-hydroxycorticosterone and aldosterone.

The biosynthesis and secretion of aldosterone by the mammalian adrenal cortex are regulated by a control system which—from a physiological as well as from a biochemical point of view—appears to be more complex than the different hypothalamic-pituitary-peripheral gland axes which control the production of other steroid hormones (Fig. 1).

STIMULATORS OF AN EARLY BIOSYNTHETIC STEP

A number of different agents promote the production of aldosterone by a rapid, direct action on the zona glomerulosa of the adrenal cortex during short-term incubation or perfusion experiments (for a review see [1]). They primarily facilitate an early biosynthetic step, i.e. one of the reactions involved in the conversion of cholesterol to pregnenolone. Angiotensin II, potassium ions and serotonin act exclusively on the zona glomerulosa of the rat adrenal cortex, whereas ACTH also stimulates steroidogenesis in the inner zones[2, 3]. There is some evidence that these agents act through adenyl cyclase. They stimulate the formation of cyclic AMP[4]; their steroidogenic effect is imitated by cyclic AMP[3] and is blocked by procaine. The activity of these stimulators depends on the presence of calcium and is inhibited by ouabain and cycloheximide[1].

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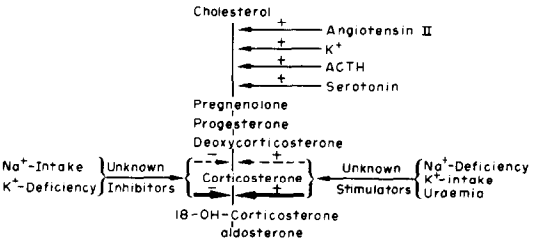

Fig. 1. Regulation of aldosterone biosynthesis in the rat. From [23].

VARIABLE STEROIDOGENIC RESPONSE TO STIMULATORS

The increase in corticosteroid output by incubated rat adrenal tissue in response to these stimulators is quantitatively and qualitatively variable and depends on the sodium and potassium balance of the animals from which the tissue is taken[3, 5]. An example is given in Fig. 2. In capsular adrenals ("zona glomerulosa") of normal rats, serotonin, potassium ions and ACTH mainly stimulated the production of aldosterone and had a smaller effect on corticosterone and deoxycorticosterone outputs. In capsular adrenals of rats which had been kept on a potassium-deficient diet for two weeks, these agents had no significant effect on aldosterone production, but elicited a strikingly increased response in deoxycorticosterone output.

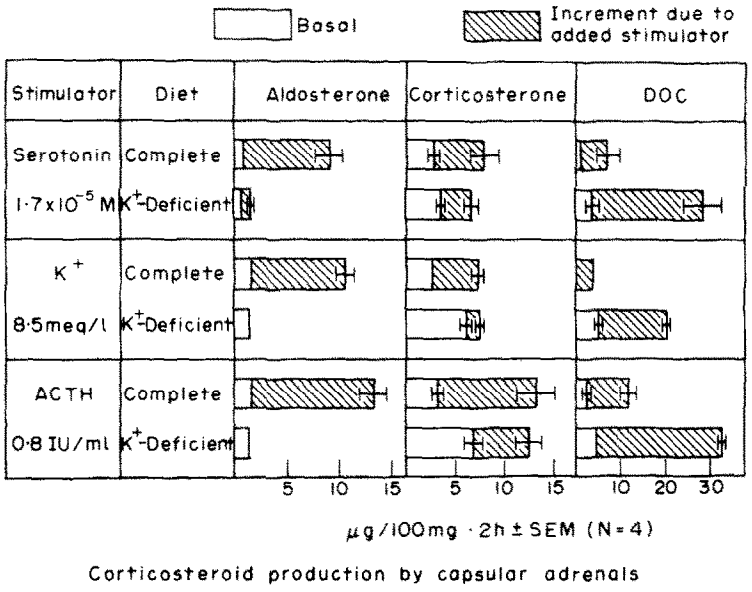

Fig. 2. Production of aldosterone, corticosterone and deoxycorticosterone (DOC) by capsular adrenals of rats kept on a normal or on a potassium-deficient diet for two weeks. White bars represent base-line corticosteroid production, shaded bars increments due to added stimulators. Mean values of two experiments ($N = 4$) ± standard error of the mean. Data from [3].

Assuming that there is just one type of zona glomerulosa cell and just one biosynthetic pathway, the varying response in aldosterone and deoxycorticosterone outputs could be due to alterations in the activity of the enzymes involved in the conversion of deoxycorticosterone to aldosterone, i.e. zona glomerulosa 11β-hydroxylase, 18-hydroxylase and 18-hydroxydehydrogenase.

ALTERATIONS IN THE ACTIVITY OF ENZYMES INVOLVED IN THE FINAL STEPS OF ALDOSTERONE BIOSYNTHESIS

Dietary sodium restriction or sodium depletion leads to an increase in the activity of one or both of the enzymes necessary for the conversion of corticosterone to aldosterone, i.e. zona glomerulosa 18-hydroxylase and perhaps 18-hydroxydehydrogenase of the rat, dog and sheep adrenal[2, 5–9]. Increased conversion of corticosterone to aldosterone can also be induced in rats by potassium loading[10]. Two weeks of potassium restriction caused a striking decrease in the

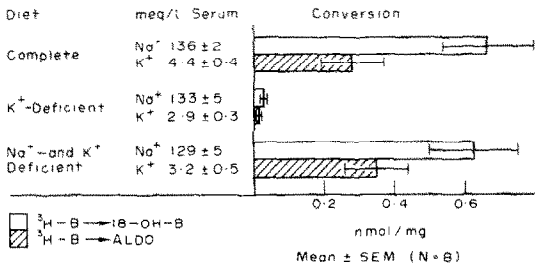

Fig. 3. Effects of two weeks of different diets on serum electrolytes and on the conversion of tritiated corticosterone (^{3}H-B, 300 nmol per flask) to 18-hydroxycorticosterone (18-OH-B) and aldosterone (ALDO) by incubated rat capsular adrenals. Mean values of 4 experiments. From [17] and [23].

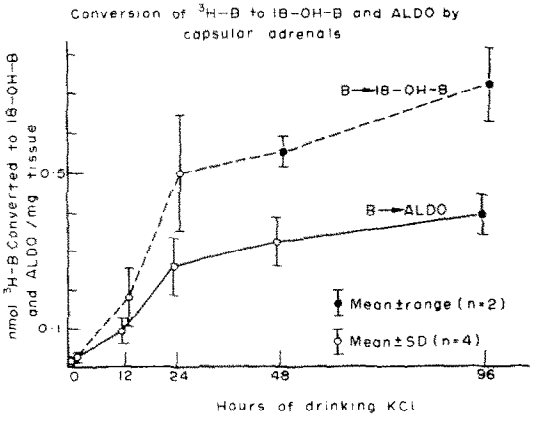

Fig. 4. Influence of time of resumed potassium intake on the conversion of tritiated corticosterone (^{3}H-B, 320 nmol per flask) to 18-hydroxycorticosterone (18-OH-B) and aldosterone (ALDO) by capsular adrenals. Rats were kept on a potassium-deficient diet for two weeks and then received a KCl-sucrose solution as drinking fluid for increasing periods of time. Data from [11].

capsular adrenal conversion of added corticosterone to 18-hydroxycorticosterone and aldosterone (Fig. 3),[11] as well as a much smaller decrease in capsular adrenal 11β-hydroxylase activity[12]. The observed alterations in zona glomerulosa 18-hydroxylase activity seem to be due to changes in enzyme content rather than to activation or inactivation of the enzyme or to the availability of coenzyme[7, 11]. They occur rather slowly in the course of days or at least several hours. When potassium chloride was added to the drinking fluid of potassium-deficient rats, it took 24 h until their capsular adrenals converted labelled corticosterone to 18-hydroxycorticosterone and aldosterone at a normal rate (Fig. 4, [11]). Similar alterations in zona glomerulosa 18-hydroxylase activity have as yet never been induced *in vitro*. Thus, at present no direct information is available about a possible physiological mechanism of mediation.

RENIN-ANGIOTENSIN

Experimental renovascular hypertension induced in rats by unilateral renal artery constriction resulted in an elevated plasma renin activity and a small increase in the conversion of tritiated corticosterone to aldosterone by incubated adrenal tissue[3]. Treatment of dogs with renin for four days led to a significant increase in the adrenal mitochondrial conversion of corticosterone to aldosterone[14]. However, at least in the rat, the renin-angiotensin system cannot be the only or even the predominant regulator of the final steps of aldosterone biosynthesis. Uraemia following bilateral nephrectomy caused a marked increase in the conversion of corticosterone to aldosterone[5, 15]. A high plasma renin activity which is found in potassium-restricted rats[16] does not prevent a marked fall in the zona glomerulosa 18-hydroxylase activity.

POTASSIUM

In addition to the effects of variations in potassium intake on the final steps of aldosterone biosynthesis, a high plasma potassium may mediate the increased zona glomerulosa 18-hydroxylase activity due to uraemia following bilateral nephrectomy[5, 15] or to sodium deficiency[5, 10]. However, capsular adrenals of rats which had been kept on a sodium- and potassium-deficient diet for two weeks and which were hypokalaemic converted tritiated corticosterone to 18-hydroxy corticosterone and aldosterone at a normal rate (Fig. 3)[17]. Moreover, the decrease in apparent capsular adrenal 18-hydroxylase activity induced by potassium restriction was partially reversed by a subsequent combined sodium and potassium restriction[17]. Thus neither the plasma potassium concentration nor the total body potassium can be the only regulator of the final steps of aldosterone biosynthesis.

SEROTONIN

In potassium-restricted rats, treatment with 5-hydroxytryptophan (i.e. a prescursor of serotonin) or with reserpine (i.e. a drug which releases stored serotonin from the blood platelets) for two days resulted in significant increases in the capsular adrenal conversion of tritiated corticosterone to aldosterone and 18-hydroxycorticosterone (Fig. 5). However, reserpine-treated rats hardly consumed any food or water. Control experiments showed that water restriction but not fasting also results in a stimulation of the final steps of aldosterone biosynthesis in potassium-deficient rats (Fig. 6). Thus the effect of reserpine may have been partly due to dehydration, which is also a potent stimulus of the renin-angiotensin system[18].

PITUITARY GLAND AND ACTH

Six days after hypophysectomy without any replacement therapy, capsular adrenals of rats kept on a sodium-deficient diet converted tritiated corticosterone to aldosterone and 18-hydroxycorticosterone at the same rates as capsular adrenals of intact sodium-restricted rats[19]. On the other hand, treatment of intact as well as hypophysectomized rats with a maintenance dose of ACTH resulted in significant decreases in the apparent zona glomerulosa 18-hydroxylase activity. Hypophysectomy with or without ACTH treatment did not impair the restoration to normal of the conversion of tritiated corticosterone to 18-hydroxycorticosterone and aldosterone in response to resumed potassium intake by potassium-deficient rats[19]. Thus, neither the maintenance nor the induc-

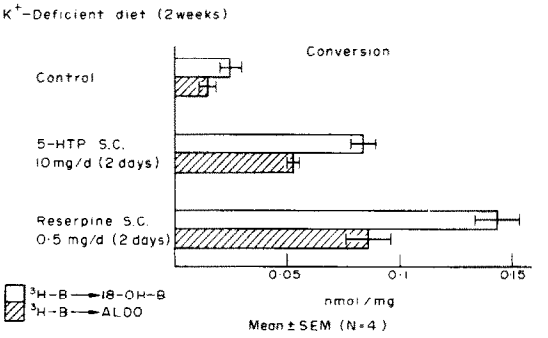

Fig. 5. Effects of treating potassium-deficient rats with DL-5-hydroxytryptophan (5-HTP) or reserpine on the capsular adrenal conversion of tritiated corticosterone (^{3}H-B, 300 nmol per flask) to 18-hydroxycorticosterone (18-OH-B) and aldosterone (ALDO). Mean values of two experiments. For methodology see [11].

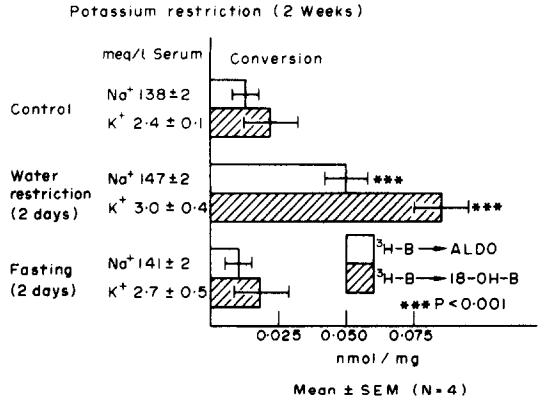

Fig. 6. Effects of water restriction or fasting on the serum electrolytes and the capsular adrenal conversion of tritiated corticosterone (^{3}H-B, 300 nmol per flask) to aldosterone (ALDO) and 18-hydroxycorticosterone (18-OH-B) in potassium-deficient rats. Mean values of two experiments. For methodology see [11].

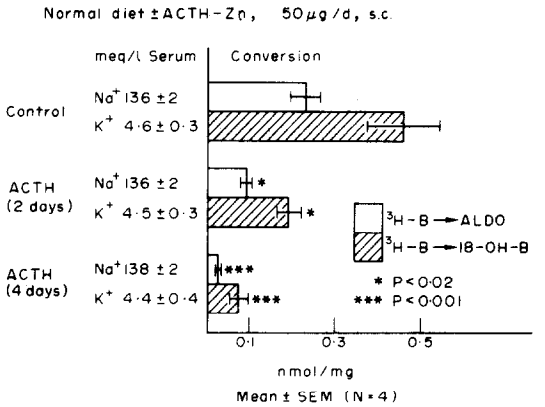

Fig. 8. Effects of treating rats with pharmacological doses of ACTH on serum electrolytes and on the capsular adrenal conversion of tritiated corticosterone (^{3}H-B, 300 nmol per flask) to aldosterone (ALDO) and 18-hydroxycorticosterone (18-OH-B). Mean values of two experiments. For methodology see [11].

tion of an increased activity of the enzymes involved in the final steps of aldosterone biosynthesis depends on a functioning pituitary gland. In rats kept on a potassium-deficient diet, hypophysectomy caused an eight-fold increase in the apparent capsular adrenal 18-hydroxylase activity (Fig. 7[19]). This may have been due to the absence of ACTH, since it was partly prevented by ACTH maintenance therapy.

ACTH, which is a potent stimulator of aldosterone production in short-term experiments, acting at an early biosynthetic stage, has a long-term inhibitory effect on the final steps. After two days of treatment of rats with a pharmacological dose of ACTH, the capsular adrenal conversion of tritiated corticosterone to aldosterone and 18-hydroxycorticosterone fell to 40% of normal, after 4 days to 15% (Fig. 8). However, the alterations in capsular adrenal endogenous corticosteroid output due to ACTH treatment were different from those induced by potassium deficiency. Thus, the base-line and serotonin-stimulated deoxycorticosterone outputs were diminished in capsular adrenals of ACTH-treated rats but markedly increased in

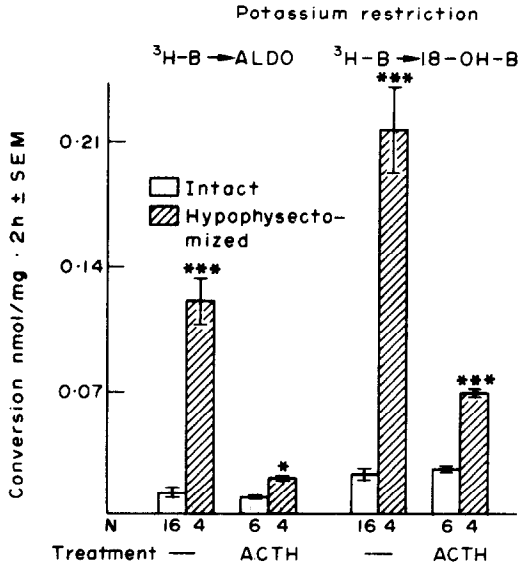

Fig. 7. Effects of hypophysectomy, ACTH treatment or both on the conversion of tritiated corticosterone (^{3}H-B, 300 nmol per flask) to aldosterone (ALDO) and 18-hydroxycorticosterone (18-OH-B) by capsular adrenals of potassium-deficient rats. Rats were hypophysectomized on day 9 of dietary potassium restriction. Synthetic ACTH-Zn was subcutaneously injected on days 9 (0·2 mg/kg), 11 and 13 (0·1 mg/kg). Incubation was on day 15. Asterisks denote the statistical significance of the difference from the respective control value (untreated rats) as calculated by t tests: $*P < 0{\cdot}005$; $***P < 0{\cdot}001$. From [19].

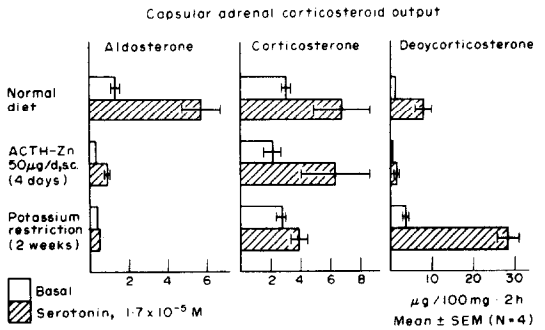

Fig. 9. Effects of treatment with a pharmacological dose of ACTH or of potassium restriction on the output of endogenous corticosteroids by rat capsular adrenals incubated under base-line conditions or with added serotonin. Mean values of two experiments. For methodology see [3].

capsular adrenals of potassium-restricted animals (Fig. 9). In additional experiments, capsular adrenals of ACTH-treated rats were incubated with substrate amounts of tritiated deoxycorticosterone (Fig. 10). The conversions to aldosterone and 18-hydroxycorticosterone were decreased, whereas the conversions to corticosterone and particularly to 18-hydroxydeoxycorticosterone (18-OH-DOC) were strikingly increased. 18-OH-DOC is a typical product of the zona fasciculata. Thus, the long-term effects of ACTH on the zona glomerulosa cell may include a transformation to a zona fasciculata-type of cell.

CONCLUSIONS

Available experimental evidence indicates that the final steps of aldosterone biosynthesis, in particular the 18-hydroxylation of corticosterone by the zona glomerulosa of the rat adrenal cortex, are regulated by a complex and multifactorial control system. Some of the mediators involved may be identical with known stimulators acting on an early biosynthetic step. Among them, the plasma potassium concentration appears to be a very important but not the only regulator. Additional stimulators may include angiotensin II and serotonin. By contrast, ACTH decreases the conversion of corticosterone to aldosterone and 18-hydroxycorticosterone. This could explain why exogenous ACTH loses its aldosterone-stimulating effect in man after one to two days of continued administration[20–22].

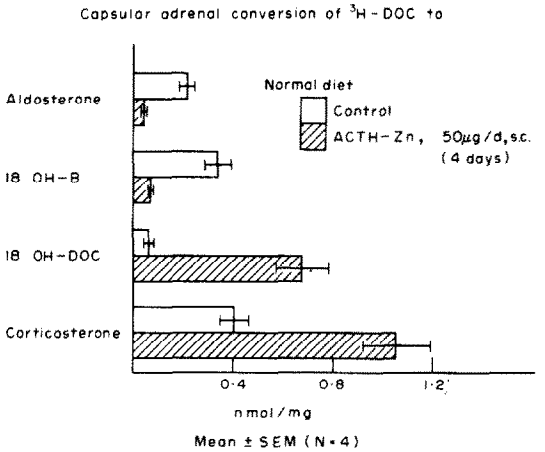

Fig. 10. Effects of treatment with a pharmacological dose of ACTH on the capsular adrenal conversion of tritiated deoxycorticosterone (^{3}H-DOC, 300 nmol per flask) to aldosterone, 18-hydroxycorticosterone (18-OH-B), 18-hydroxydeoxycorticosterone (18-OH-DOC) and corticosterone. Mean values of two experiments. For methodology see [11].

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DISCUSSION

Tait, J. F.:

First, in line with Dr. Müller's conclusion that intracellular potassium may not be the only factor in controlling aldosterone secretion, Dr. Mendelsohn working in our laboratory with purified glomerulosa cells has found two situations where there is complete dissociation of intracellular potassium and aldosterone secretion; one with serotonin stimulation where the intracellular potassium does not change even with maximum steroid outputs and the other with ouabain inhibition. He can apply a dose of ouabain which lowers intracellular potassium significantly but does not lower the steroid output. Secondly, I think in one of your slides you showed the time course of the increase in the conversion of B to 18-hydroxy-B and B to aldosterone which I think is very vital. information. I wondered if at the same time you measured the B production from the glomerulosa? This is perhaps the critical point.

Müller:

No, we haven't.

Adlercreutz:

I would like to ask you if you need the whole ACTH molecule to exert this effect, or if only a part of it could have the same effect.

Müller:

All these experiments have been carried out with the synthetic ACTH with 24 amino acids.

Kolpakov:

I should like to ask you about the mechanism of action of ACTH inhibition on the transformation of corticosterone to aldosterone. Your slide showed that ACTH stimulated transformation of deoxycorticosterone to corticosterone and inhibition of transformation of corticosterone to aldosterone. Is that right? What is the mechanism of action?

Müller:

I don't know exactly what the mechanism is, but it seems that the zona glomerulosa cell is in some way converted to a functional zona fasciculata type of cell. A similar phenomenon is found in adrenal regeneration hypertension where a zona fasciculata type of adrenal cortex develops from the capsule and the remaining zona glomerulosa cells. I think Dr. Birmingham has some comment on that point.

Birmingham:

Actually, histologically, the regenerated glands have tissue that looks like zona glomerulosa, but under the influence of ACTH or what have you, certainly the corticosterone, 18-hydroxy-DOC and also DOC in the beginning goes up and aldosterone is relatively suppressed.

Müller:

I don't know whether this is the only mechanism of the inhibitory effect of ACTH. In some experiments, it looks as if alterations in serum potassium or in overall potassium balance could also be partially responsible for the decrease in aldosterone output.

Tait, S.:

Have you examined the adrenal glands of such animals by electron microscopy to see whether any changes have occurred in the zona glomerulosa cells, such as alterations in the mitochondial cristae from the typical tubular to the vesicular type characteristic of zona fasciculata cells.

Müller:

This part of the work is being done now by a pathologist but I don't know the results yet.

Vinson:

I was interested in the effect of potassium depletion giving apparently increased secretion of deoxycorticosterone, suggesting that potassium may have a stimulatory effect on 11β-hydroxylation or 18-hydroxylation possibly of DOC. Do you have any more specific information about that? Have you done any experiments in cases of potassium depletion using tritiated DOC as a precursor for example?

Müller:

Yes, we have, but with the impure zona glomerulosa tissue preparation, there was no apparent decrease in the conversion of tritiated DOC to corticosterone and 18 OH-DOC. Only the conversion of tritiated DOC to aldosterone and 18-hydroxy-B was significantly decreased.